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EIGHTH TECHNICAL ALFALFA CONFERENCE - PROCEEDINGS

Held at Colorado Springs, Colorado, February 6, 1963

THE EIGHTH TECHNICAL ALFALFA CONFERENCE reported here was held February 6, 1963, in Colorado Springs, Colorado, in conjunction with the Annual Convention of the American Dehydrators Association. It was attended by forage growers and processors, equipment manufacturers, manufacturers of feedstuffs, feed lot operators, and research personnel in State Agricultural Experiment Stations and the U. S. Department of Agriculture.

Staff members engaged in forage investigations in the Western Utilization Research and Development Division of USDA reported research dealing with the efforts to improve forage processing methods and stressed the need for greater emphasis in this area of research. Members of the Crops Research Division of USDA reported recent agronomic advances in alfalfa production. Representatives of industry spoke on the value of dehydrated alfalfa in improving the marketability of poultry products. Workers from universities and State stations reported various aspects of research on forages as related to animal nutrition with special emphasis on the role of the physiologically active constituents. A representative of Nebraska's State Department of Agriculture and Inspection discussed that Department's efforts to upgrade dehydrated alfalfa feed products under provisions of a special grant to the Western Division in Albany, California.

The conference was co-sponsored by the Western Division and American Dehydrators Association. The program was developed by George O. Kohler, Chief, Field Crops Laboratory, Western Division, and Richard L. Kathe, Executive Vice President, American Dehydrators Association, in cooperation with other groups. K. W. Taylor, Engineer in the Fields Crops Laboratory of the Western Division, reviewed and technically edited this report.

This report was compiled in the Western Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture, Albany 10, Calif. Copies are available on request.

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PROCEEDINGS OF THE EIGHTH TECHNICAL ALFALFA CONFERENCE

A LAYMAN LOOKS AT ALFALFA RESEARCH

Richard L. Kathe, Executive Vice President
American Dehydrators Association, Kansas City, Missouri

Several of you are aware that I was employed by American Dehydrators Association last April to replace retiring Joe Chrisman as Executive Vice President. I don't think anyone believed I would pretend to replace him as a person expert in the science and production of dehydrated alfalfa. Nevertheless, I am charged with the coordination of all aspects of the Association's program, including co-sponsorship of alfalfa research at publicly financed institutions. This duty requires that I properly encourage activities deemed worthwhile. It demands some judgment upon research now being conducted and that which is proposed.

My background in science is meagre compared to that of scientists active in research. Courses in agronomy, chemistry, bacteriology, soils and geology were well taught but inadequately received and retained. Therefore, to the scientists I extend a personal plea for your helpful counsel.

For many years, American Dehydrators Association has been greatly assisted by the volunteer service of outstanding scientists from public institutions and industry who comprise our Research Council. Through their unselfish efforts, and the willingness of our Association to provide funds, many excellent research projects have been carried out. This will be described by Dr. William Kehr, our next speaker.

All of us are faced with one constant problem ... where we will go from here ... what will be our future course of action. The future is, of course, dependent upon the present, determined by the past. So, may I presume to offer a brief sketch of my impression of the historical background.

Biochemical research seems to run in cycles. At the beginning of this century investigators were preoccupied with nutritional combinations of natural feedstuffs and byproducts from industries processing grains. When values were proved, scientific activity entered a second phase. It turned to examination of the specific nutrient components in these feedstuffs. Several vitamins were discovered in the 20's and 30's. A parallel activity was the study of minerals and their best combinations.

The 40's and early 50's saw the pendulum of science swing to research directed toward a re-examination of natural feedstuffs and improved processing. Alfalfa was a good example. Large amounts of research on dehydrated alfalfa of higher protein and carotene were conducted. As feeding values were proved the pendulum swung again and investigators turned their energies toward synthesizing

nutrients. New vitamins, hormones, and other chemical compounds with names long enough to choke a horse began to appear in the feed trade news and to replace terms like "grass juice factor," "animal protein factor," and "unidentified growth factor."

At what point are we in this theoretical cycle? So long as chemists are engaged in research, we will have a dual effort of isolating compounds and determining their specific role in nutrition. These efforts may be followed by synthesis if that is economically feasible. The question somewhat deciding future success of the alfalfa industry is, which of these efforts will have greatest emphasis? At this point in history producers of the natural ingredients turn to scientists and say, "What have you done for us lately?" Some groups, such as alfalfa dehydrators, have made big strides in improving nutritional value of their delivered products and are now in a marketing phase of their industry cycle. But the pressure is on and I don't envy the scientists' role. All industries and public institutions are in a price-cost squeeze. The old shotgun approach, when money was more plentiful after World War II, won't work today. Everyone has to sight his rifle on clearly defined targets. The after-hours work schedule is taking its toll of personal happiness and leisure, but worldwide competition is forcing us all the way.

Where do I think this leads those of us interested in alfalfa? Perhaps into three channels uniting in one main stream. Naturally, the first channel is to find more of the unknown nutrients still locked in the mystery that is alfalfa, and to determine the feeding reaction of these nutrients. The second channel will be to grow and produce an alfalfa product with optimum amounts of the desired nutrients; for example, to produce a higher-protein dehydrated alfalfa for dairy calves, or a high or low coumestrol "dehy" for beef or sheep in various stages of their life cycles, would require incredible amounts of plant genetics and agronomy research, not to mention improvements in dehydration, storage and marketing.

Nevertheless, trends in commercialization and specialization of livestock and poultry production dictate feed ingredients more closely aimed at better targets. Economical competition in the ration may force this development, as well as the third channel of research.

The third channel will be the applied research of refining existing knowledge of alfalfa utilization. Feeding tests will be conducted to determine the competitive position of alfalfa versus other ingredients on specific nutritive questions. Our industry must have answers on many of these questions in the immediate future.

Thus, we have three types of research: the basic problem of finding more of the useful nutrients within alfalfa, the genetic, agronomic and production problem of producing a more nutritive product and the marketing problem of improving the competitive position, through refinements of utilization knowledge, in comparative tests.

It's obvious that these three kinds of research are closely dependent upon each other. It is apparent that close liaison is essential. This Technical Conference serves as an exchange of information, and has some function in

coordination of effort. The ADA Research Council is limited by a relatively small budget, in one sense, in the amount of work which can be stimulated. Possibly the future research on alfalfa may be coordinated by a national committee or council concerned with all three aspects of research and representing all of the resources available.

Now that I have raised a question which has no immediate answer, it may be a good time to stop. However, let me repeat that I'm most anxious for the help of scientists and seek your counsel on our future course. On behalf of our industry, may I extend our sincere thanks for the excellent work you have done and will do in the future.

RESEARCH SUPPORTED BY AMERICAN DEHYDRATORS ASSOCIATION, 1949 to 1961

ADA Research Council, W. R. Kehr,[1/] Chairman

Research at experiment stations demonstrated in the early 1920's that the carotene and protein of alfalfa were lost to a large degree in common haying methods. Physical losses occurred from shattering. Chemical losses occurred from leaching, oxidation, and respiration. The dehydration industry was born from a need to preserve the constituents vital to livestock production. Teamwork among scientists has nourished the success of this industry.

As the industry was maturing its leaders realized that facts were needed to maintain present markets and to expand into new markets. It was realized that no competitive industry could survive on false premises. Facts were needed on which successful advertising could be based. Thus purposes for an ADA-supported research program through grants-in-aid were clear. Facts were needed on which advertising and sales could be built. There was need for research to learn more about every facet of the industry including production, processing, animal nutrition, and marketing. There was need for information clearly stated in readily available and usable form for every segment of the industry.

Before one can intelligently plan where he is going, he must look at where he has been and where he is today. The accomplishments of today, however, do not reduce the need of research for tomorrow. The need for continued research on food production is evident when one considers population increase, reduction in acres available for tillage and soil erosion losses.

[1/] Research Agronomist, Crops Research Division, ARS, USDA, Lincoln, Nebraska.

Thus a review was made of all ADA-supported research from the initial projects in 1949 to those proposed for support in 1963-64. This review revealed research that helped bring the industry from infancy to maturity, from a chemically unstable hard-to-handle product to a modern product designed to fit the age of automation in the feed industry, from haymaking to a packaged product geared to keep animal products on the table of the best-fed people in the world. This review revealed a self-help program, "operation bootstrap," skillfully designed and carefully executed cooperatively through the hands of industry, and of research personnel at various state, federal, and private research institutions including the ADA Research Council, with results directly transmitted into action programs. This is truly the type of program which has made our country strong and of which every participant can be proud.

Research Projects

Research has been conducted at 22 locations. These include experiment stations in Arizona, California, Colorado, Connecticut, Florida, Illinois, Indiana, Iowa, Kansas, Michigan, Missouri, Montana, Nebraska, New York, Oklahoma, Saskatchewan (Canada), South Dakota, Texas, Utah and Washington. The other locations are the USDA Western Regional Research Laboratory at Albany, Calif., and the Wisconsin Alumni Research Foundation.

Research support was divided into 9 subject-matter areas. These areas and the percentages of the funds spent in each from 1949 to 1963 are as follows:

	%		%
Agronomic	13.4	Economics	2.0
Antioxidants	3.8	Poultry	22.8
Beef	15.7	Sheep	11.2
Chemical	11.3	Swine	7.3
Dairy	12.5		

In some cases, research could be placed in two or more categories.

A program of reporting somewhat detailed results from projects on an annual basis was initiated in 1961 at the summer meeting of the Board of Directors. Results from the 1961-62 program were presented at the 1962 convention and were documented in the brochure "Harvest of Ideas." Results from the 1962-63 program were presented at the 1963 Council Meeting. Beginning with the 1963 convention, grant recipients were requested to prepare a summary paragraph for ADA membership usage, in addition to a detailed progress report for Council review. This procedure was instigated to expedite transmittal and use of information.

This progress report is a brief résumé of information obtained from 1949-1961 grants and is based on reports presented to previous councils. Official publication, including full interpretation of results, is reserved for the investigators. ADA files of previous Council members from Nebraska, Drs. Severson, Baker and Frolik, were received in 1959. Present files have, with few exceptions, project outlines and reports on every project supported. The exceptions are a few projects supported for one year in early years of the program.

Project Results

For brevity, these results are reported on a subject-matter basis without regard for location of work, in general.

Agronomic. California studies on the effects of plant nutrition and harvest procedures are reported in their Experiment Station Bulletin 784 entitled "Controlling alfalfa quality." The studies were extensive. This bulletin was mentioned in Dehy Review and other media. It is available upon request from the California station.

Alfalfa fertilizer requirements for the Rio Grande Valley of Texas revealed exceptionally favorable response to phosphate. The Bray method for determining phosphate requirement could be modified for use with alkaline soils.

Studies of the root system of sub-irrigated alfalfa revealed two important regions for water and nutrient sorption--the moisture fringe above the water table and the surface soil. In land continuously cropped to alfalfa, phosphate content was reduced to the 11-foot level. Available nutrients in the soil substrata must be evaluated before reasonable fertilizer recommendations can be made. This was some of the initial work on the use of radioactive chemicals.

Antioxidants. Studies on several antioxidants, including Santoquin, showed low toxicity when used in recommended amounts. The work on antioxidants is well known and was a turning point, with inert gas storage, in the preservation of quality.

Beef. "Dehy" increased rate of gain and decreased feed costs in several studies. Several studies indicated the possibility that estrogenic substances were in "dehy." Carotene content of the alfalfa-containing supplement was found to be the important factor when roughage of low quality is used. The use of "dehy" improved performance of gestating-lactating heifers consuming a ration high in wheat straw.

Chemical. A method was developed for preparing a carotene concentrate from dehydrated alfalfa meal. The concentrate contained, among other things, carotene, a wax, sterol, and fat. In another study of numerous methods the hot dilute alkali method was most effective in extracting protein. The method removed from 87 to 100 percent of the protein. The work on coumestrol is well known and was reported at the 1962 convention and the 7th Technical Alfalfa Conference.

Dairy. One study emphasized (a) the importance of higher carotene intakes in winter feeding following drouth, poor pasture conditions or during poor roughage feeding and (b) that "dehy" contributes significantly to the tocopherol status of the calf. In another study (a) cows that received "dehy" at a level of 0.5 lb./100 lbs. of body weight consumed more feed and produced more milk and butterfat than when on a normal ration and (b) cows that were fed "dehy" at 75 or 100 percent of the roughage intake showed a marked reduction in butterfat percentage and depressed rumination. Results from a third study showed that the inclusion of small amounts of "dehy" in the ration had little

or no effect on total milk production, body weight change or total roughage dry matter intake of lactating Guernsey cows. A slight nonsignificant increase in average persistency of milk production was observed.

Economics. One grant-in-aid contributed to well-known publications which are widely used. These are: "Marketing dehydrated alfalfa," USDA Marketing Res. Report No. 258, 1958, by Reed, Schruber and Cathcart, "Costs and efficiency in alfalfa dehydrating plants," Kansas Ag. Exp. Sta. Tech. Bul. 124, 1962, by Reed.

Poultry. Growth stimulation or improvement due to unidentified factors in "dehy" was reported from several studies. The "dehy" factor was apparently different from that of other materials and gave the greatest and most consistent response. Growth inhibitory effects were reported from several studies when chick rations contained over 20 percent of "dehy." In one study of rations containing 10 percent "dehy," great variability was expressed between samples but about 80 percent of the samples examined caused no depression. Inhibitory effects were found among sun-cured as well as dehydrated meals. Results suggested that environmental factors in the growing season influenced nutritional properties. In other studies losses of carotene from alfalfa were not materially affected by other components in the ration, whereas this was not true with carrot oil-supplemented diets. Alfalfa was found to be a good source of unidentified factors required for the maintenance of fertility and hatchability of eggs from turkey hens where breeders were maintained for a period longer than 11 weeks.

Sheep. One study reported that none of the lambs which received "dehy" in the ration were afflicted with urinary calculi. Additional experiments are in progress on this observation. Another study concluded that "dehy" can be used as a range supplement to replace part of the grain and protein supplement.

Swine. Results from a current project were reported at conventions in 1962 and 1963.

Discussion

It must be recognized that much early work on "dehy" was done with products different from today's modern product which, by and large, results from treatment with an antioxidant and is stored under inert gas. The Council now asks recipients of grants to analyze all lots of "dehy" used in their experiments, promptly on receipt, for protein, fiber, ash, carotene and coumestrol and to repeat carotene analyses at least every 30 days until the supply is exhausted or the experiment concluded.

The grant-in-aid program means different things, depending on individual and group interests.

ADA members make a financial contribution which is substantial. Council Chairman Bird estimated in 1960 that the $189,637 of ADA money spent on research from 1949 to 1959 actually was worth $455,100. This thinking was based on the fact that a grant is invariably matched, or the grant amount is exceeded, by other funds from the sponsoring institution. Utilizing the same ratios suggested

by Bird, the expenditure of about $250,000 from 1949 to 1963 bought about $600,000 worth of research. Looking at the cost of financing research in another way, recent estimates indicate a cost of $25 to $30,000 per year for one project at one institution. Several investigations may be under one project. This includes salaries, physical facilities and operating funds. Thus, use of funds to support research through grants gives maximum mileage per dollar. Research costs; but it pays too, as every member knows. Some research facts can be used to put out fires that threaten markets. Other research facts can be used to build fires that create markets.

Research people usually have more ideas for research that can be financed with appropriated funds. They tend to have more projects in progress than appropriated funds will adequately support. Research grants are not used to make money for the recipient. Grant-in-aid funds are used to conduct research on projects of mutual interest to the grantor and the grantee. Supplementary funds in the form of grants often carry the researcher "over the hump" on another phase of investigation. ADA-supported grants have been and are made on carefully planned work to some of the best investigators available. The Council consists of active investigators. Council suggestions are usually welcomed by grantees and incorporated in the project. At times a project is rejected unless Council suggestions are incorporated.

The public gains from research. High-quality foods are available on store counters. We as a nation spend a smaller portion of our salary for food than many other nations.

Every state and seemingly every community advertises to attract new industries to bolster its economy. Any time industry and research people from public institutions team up to conduct research that assists established industries, such as the dehydration industry, the entire public stands to benefit. The cooperation and friendship which exists between ADA members and research personnel is a tribute to mutual interests.

The résumé reported here and those presented at the 1962 and 1963 ADA conventions have been and are currently supplemented in detail by various publication media. The media include Alfalfa Abstracts, Dehy Review, bulletins, reprints of articles published in many forms and the "12-month summer." Preliminary results are usually available as stages of research are completed. Translation of results with clarity and brevity into pellet-sized packages takes additional work and time. Final publication of results involves much work and time. Patience is of course necessary.

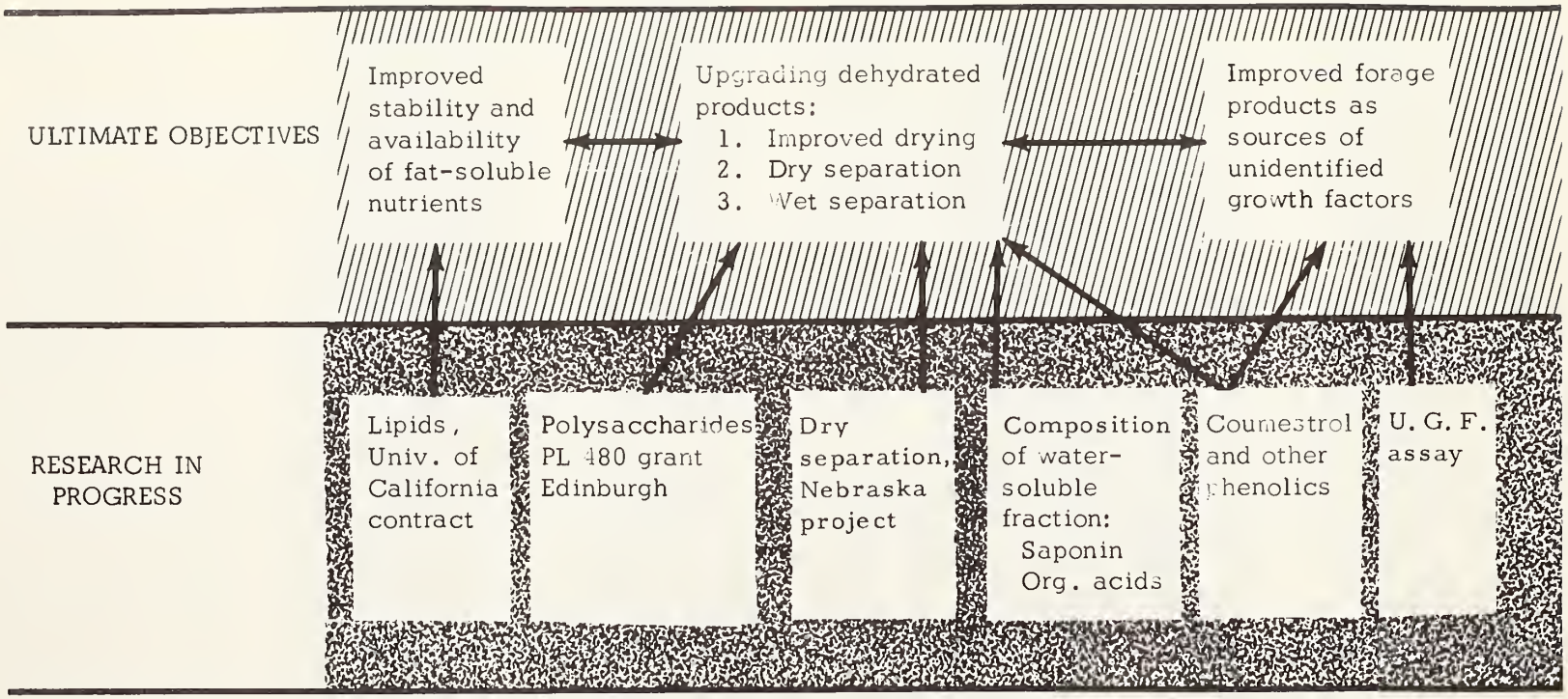

Figure 1. WRRL utilization research on forages.

CURRENT FORAGE RESEARCH AT THE WESTERN REGIONAL RESEARCH LABORATORY

George O. Kohler
Western Regional Research Laboratory, USDA, Albany, California

I shall start by giving you a sketch in Figure 1 (opposite page) of our program to show ultimate objectives and the relationship of current work to these objectives. I shall then describe selected items of progress which have been made since the last Technical Alfalfa Conference held a year ago last July. These descriptions will be incomplete, since each block in Figure 1 would well take an hour or so of discussion without going into excessive detail, at least from the standpoint of the scientist.

The "ultimate objectives" of our program are shown across the top of the figure. The first box is "Improved stability and availability of fat-soluble nutrients." Why work further on stability when we already have inert gas storage and ethoxyquin? Although much of the "heat" is off the stability problem in domestic markets, the emergence of huge export potentials requires that we take another look. It would be difficult to apply inert-gas storage through the complex distribution channels to the Far East, South America, and Western Europe. Without ethoxyquin I believe we would not have a chance for these markets. While ethoxyquin reduces losses of carotene, xanthophyll, and vitamin E to a third of normal losses, that remaining third could well become a major factor for the export market where very long "in transit" times are unavoidable. I believe that the U. S. productive efficiency through large-scale operations, high capital investment, and know-how will favor dollar-wise competition with anyone, but we would certainly be better off in building large export markets if we could achieve a 100 percent stable product. The additional funds allotted to forage utilization research from the 1963 budget will permit more work in this area.

The next objective shown in Figure 1 is to upgrade the utility and value of dehydrated products. I refer especially to correcting certain inherent compositional defects of alfalfa which, in current products, tend to limit its use. The dehydrated forage industry is built around use of its product as a nutrient concentrate. Yet about 50 percent, including the cellulose, hemicellulose, pentosans, silicic acid, and lignins are essentially indigestible to poultry and swine. Ruminant animals can use some of the fibrous constituents but even here the lignin of "dehy" prevents complete utilization of carbohydrates. Processes are needed for separating alfalfa into low-fiber products for poultry and swine and high-fiber products for ruminant feeding. High-fiber products should be treated so as to render the cellulose and other complex polysaccharides completely digestible by ruminant animals. I first started working in this field in 1939 and even then the patent literature was cluttered up with impractical inventions. The idea is sound but no one has had the facilities and backing to carry out the necessary basic and developmental research. Enough promising progress has been made so that we feel that this is the direction which forage processing will take in the future. We are accordingly directing much of our effort into this area.

A third ultimate objective (Figure 1) is to "Improve forage products as sources of UGF" (unidentified growth factors). This might have been placed under objective No. 2, but I feel that it is so important that it should be given top priority in utilization research. I mentioned that dehydrated forages are used exclusively as nutrient concentrates. Which nutrients? Certainly not protein or carbohydrates. The feed manufacturer sees dehydrated alfalfa primarily as a concentrated source of carotene, vitamin E, and vitamin K. For specific uses where pigmentation is needed, xanthophylls of dehydrated forages are primary requirements. But if the known identified nutrients were all that dehydrated forages had to offer, the dehydration industry would be in serious trouble. Cheap competitive products have taken away much of the sales appeal of carotene, vitamin E, and vitamin K. Competition from new pigmenters is mounting. The main reason for the large and growing use of dehydrated alfalfa today is that when fed to animals it sometimes produces beneficial results which cannot be explained on the basis of known vitamin content. This "something" in forages is called UGF for unidentified growth factor, or sometimes it is termed the "grass juice" factor. This factor, or factors, is partially or sometimes completely lost during dehydration. Since no simple method for measuring it is known, the seller cannot guarantee its presence, nor can the buyer be certain he is getting a potent product. He buys the product and hopes for the best. He at least knows he is getting carotene and xanthophylls. This UGF problem presents the greatest opportunity for solidifying present markets and for building new ones, in my opinion.

I have spent a lot of time on this first part of my talk since in research as in other endeavors you need to know where you want to go before you start. If we could go directly to the objectives I have been discussing we would have strictly an applied research program, much of which might be done by industry itself. But there are too many missing pieces of basic information about constituents and interreactions that occur during processing. Accordingly, we chop the problem into pieces, which we may call limited objectives, and direct our attention to solving them. These are shown in the middle row of boxes in Figure 1.

The first box in the second line refers to lipids, University of California contract. This work is directed toward a 100 percent stable product as shown by the arrow on the figure.

Ethoxyquin was discovered to be an effective alfalfa meal antioxidant during the course of a screening program. This program was discontinued when we ran out of promising compounds to test. All our effort was then directed toward proving the safety of ethoxyquin and toward development of practical means of using it. During the period from 1956 to 1960, Dr. Harold Olcott of the University of California undertook the problem of stabilizing fish oils with antioxidants. He found that when ethoxyquin plus certain amines was added to menhaden oil, a very great stabilizing effect was obtained (Table 1). Neither ethoxyquin nor the amines had any effect if used alone. Such a relationship is called synergism. Because of this work and other basic research he was doing on autoxidation, we felt that he would be an ideal person to tackle the job of complete stabilization of alfalfa meal, and we arranged a contract for research in this field. Briefly, the results so far have shown that the amine synergists did not work on ethoxyquin-treated alfalfa meal as they did on fats

Table 1. Synergistic effect of amines in menhaden oil (Olcott)

Additive	Induction period (days)
Ethoxyquin	1
Ethoxyquin + dioctylamine	30
Ethoxyquin + trioctylamine	31
Dioctylamine	1
Trioctylamine	1

and oils. There are several possibilities as to why this is true. One of the more attractive hypotheses is that non-carotenoid alfalfa lipids are involved, since it is known that unsaturated fatty acids may affect destruction of carotenoids by co-oxidation. Since the nature of alfalfa lipids was incompletely known, the California group undertook to investigate them. Results thus far have verified the presence of large amounts of galactolipid in alfalfa and have shown that the fatty acids of alfalfa contain a surprisingly high level of linolenic acid (Table 2). Further contract work with Dr. Olcott and his group is planned and will be directed toward a study of alfalfa lipids and the changes which take place in them during processing and storage.

Table 2. Fatty acid composition of alfalfa lipid fractions (Olcott)

Acid	Hexane soluble	Galactolipids	Acetone soluble	$CHCl_3$ soluble
	Percentages of total fatty acids			
Lauric	4	--	1	tr
Myristic	tr	--	2	1
C_{15} acid	tr	--	1	tr
Palmitic	34	6	16	18
Palmitoleic	5	--	--	2
Stearic	5	2	5	2
Oleic	6	2		3
Linoleic	27	3	13	14
Linolenic	19	84	56	46

Plus at least five unidentified higher molecular-weight acids.

So much for the California contract research. Looking again at the coordination chart (Figure 1) we see in the upper left hand box that availability of fat-soluble nutrients is mentioned. In the case of vitamin E there is still disagreement as to the biological availability. The ADA has been supporting research at the Connecticut Station. We at the Western Lab have been cooperating with Dr. M. L. Scott at Cornell. Both ADA and the Western Lab have supported work at the Wisconsin Alumni Research Foundation. The low availability figures obtained at Connecticut as contrasted with the results at Cornell, Wisconsin, and Illinois cannot yet be reconciled. In the case of alfalfa xanthophylls, the results from different laboratories are pretty much in agreement. Availability figures range from 33 to 70 percent. We have been cooperating with Dr. Wendell Carlson of the South Dakota Experiment Station in attempts, thus far unsuccessful, to increase availability of xanthophylls by fine grinding of

alfalfa. Earlier cooperative work with Dr. Francis Bird indicated that the pelleting operation improved pigmentation value somewhat. There is a good opportunity to upgrade the value of alfalfa by finding means of making the xanthophylls more available.

Turning to the second box in the lower line of Figure 1 we see reference to research on alfalfa polysaccharides in Scotland under a Public Law 480 grant. While the research is already under way, we have not yet received any reports so all that I will be able to do is to describe the projected work. The polysaccharides of alfalfa constitute about 40 percent of the dry weight of the plant. The crude fiber, which amounts to about 25 percent consists of polysaccharides--but some of the original polysaccharides are not measured by the crude fiber procedure. Ruminant animals are known to be able to utilize 40 to 60 percent of the polysaccharides but poultry and swine are generally considered to be unable to digest them. Thus more information is needed on the carbohydrase enzyme systems of alfalfa which are involved in the metabolism of the structural carbohydrates including pentosans, pectins, hemicelluloses and cellulose. This knowledge will be applied to investigation of polysaccharide structure and to the binding of polysaccharides with each other and with lignin.

The problem is a difficult one, and we are fortunate that the work is being directed by Dr. E. L. Hirst, one of the world's outstanding authorities in polysaccharide chemistry.

The next box in Figure 1 shows the Nebraska project, which is directed toward dry separation of high and low protein products from dehydrated alfalfa by application of sieving, fine grinding and air classification procedures. Since Joe Chrisman will report progress this afternoon, I will say no more about it now.

Another box in Figure 1 concerns composition of the water-soluble fraction of alfalfa. While a great deal of additional research is needed, our efforts at present are limited to research on saponins and organic acids. At the Seventh Technical Alfalfa Conference, Dr. C. L. Hanson of Crops Research Division reported on cooperative studies in which we found that of the varieties tested, Du Puis showed the highest average saponin content. With the cooperation of one of the large dehydration companies we were able to obtain 400 pounds of high saponin Du Puis alfalfa. We have been working on improvement of the isolation procedure and will shortly undertake the large-scale preparation of saponin for evaluation in biological tests and as a surface active agent. Along with this work Dr. Walter of our group has been investigating the sterols of alfalfa. He has recently found that contrary to earlier reports, sterol occurs as the palmitate ester and as the glycoside as well as in the free form.

Another important group of components of alfalfa is the organic acids. These make up 5 to 10 percent of the dry weight. While some of the organic acids are known, detailed work on the effects of environmental and processing conditions on the group needs to be carried out. Dr. J. M. A. Tilley of the Grasslands Research Institute, Hurley, England, who has recently joined our group on a Kellogg Foundation Fellowship, will investigate organic acids of dehydrated alfalfa.

Next is coumestrol and other phenolic compounds. You will recall that workers at the Western Regional Research Laboratory have isolated and characterized this new estrogenic compound in work reported at earlier conferences. Development of a sensitive analytical procedure has made it possible to learn a great deal more about the factors affecting the amount of coumestrol in different alfalfas from different parts of the country. With the fine cooperation of dehydrator operators, we have been thus able, for several successive years, to obtain large amounts of highly potent dehydrated alfalfa meal. In 1961 we again set up field trials with this object in mind. The results in Table 3 show the coumestrol contents of alfalfas at different stages of growth grown at different locations. By selecting lots we were thus able to obtain 75 tons of high-potency meal from the midwest and the east. While considerable information was obtained on feeding value of high-coumestrol alfalfas by Dr. Oldfield at Oregon State and by Dr. Matsushima at the Nebraska Experiment Station, some of the results were difficult to interpret because of uncontrollable differences in fiber, lignin and protein of the high and low coumestrol alfalfas. In order to obtain uncomplicated evaluation data on large animals, we had 45 tons of the high coumestrol alfalfa processed by the scheme shown in Figure 2.

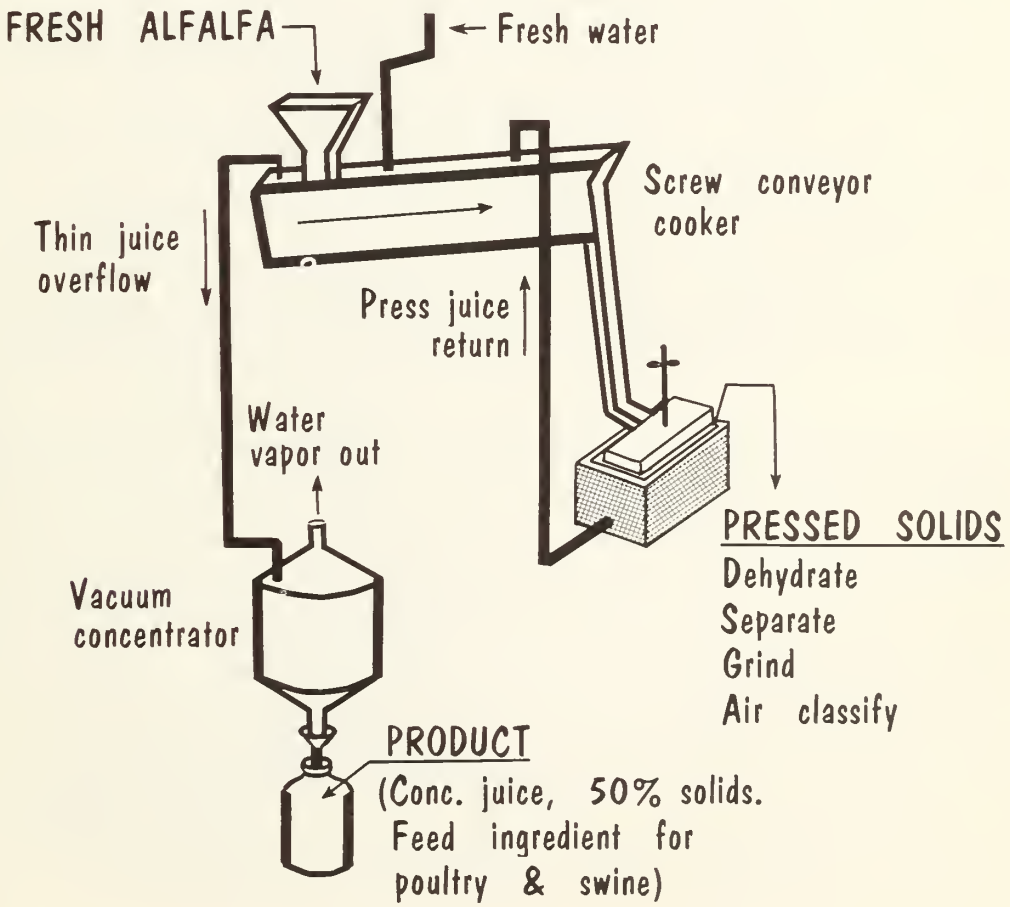

Figure 2. Process used for production of juice concentrate.

This operation yielded 2,500 pounds of a concentrate containing 5,600 ppm of coumestrol. Thus the concentrate was about 14 times as potent as the original alfalfa. This product was supplied to Dr. J. Oldfield and Dr. W. Beeson for cooperative feeding experiments on wethers and steers. Results are not yet available from the Purdue study but Dr. Oldfield will discuss his data later in the program. In our Laboratory we have been working on the composition of this concentrate from a chemical standpoint and were able to isolate about two pounds of crystalline coumestrol and lesser amounts of as yet unidentified compounds. Results of paper chromatographic tests on fractions separated by use of countercurrent distribution have demonstrated the presence of at least 125 fluorescent compounds. By countercurrent distribution and fractional crystallization at least 10 of these have been isolated in pure crystalline form. Work is progressing on further isolations and on chemical characterization of the new compounds. From discrepancies between bioassays and chemical tests, we suspect that another new estrogenic compound may be present.

Let me turn now to the subject of unidentified growth factors of forages. I have already described why we are interested in them. The limited amount of effort which we have been able to direct to it has resulted in further verification of UGF activity for the chick. Recent experiments show that alfalfa diffusion juice prepared with sugar beet pilot plant equipment (Figure 3) is presently the most reliable source of UGF we have. One fractionation step has been devised which results in removal of inert solids from the active juice fraction. Two experiments have shown that over half the activity is lost during dehydration. Whether or not dehydration conditions can be modified or pretreatments can be devised which will prevent such losses remains to be determined. We are obtaining a pilot-scale dehydrator for use in such experiments. Its capacity is 100 pounds per hour of dry product. A major problem lies in the fact that the chick assay is slow and lacks precision. The amount of increased growth obtained with chicks amounts to about 5 to 15 percent. From a practical standpoint this is a great deal but from the standpoint of a biological assay to determine effects of processing or fractionation, it is quite small.

The same problem had been encountered in the work with folic acid and vitamin B_{12}--two one-time unidentified growth factors which have now joined the ranks of known vitamins. In both cases rapid microbiological assay procedures were found which greatly facilitated and speeded investigations. Accordingly, we are using part of the additional funds allocated to forage utilization research this year to the development of a rapid bioassay for the grass juice factor. Initially, we plan to screen a wide variety of bacteria, protozoa, fungi, insects, gastropods and other lower forms of life for type of response to our alfalfa preparations known to be active for chicks. Growth stimulation in many of these organisms has already been reported in the scientific literature. We then plan to determine the potential of responding organisms for quick assay of the growth factor and to correlate the properties of growth factors found with chick growth factor. The next step will be to apply the best quick assay to isolation of the factor and to studies of the effect of dehydration on alfalfa potency. Arrangements have also been made for correlation of our findings with guinea-pig growth factor research at the University of California.

Table 3. Coumestrol in alfalfa

Date (1961)	Stage of growth	Height (inches)	Coumestrol (ppm)
Dixon, California			
May 16	bud	--	8
23	1/10 bloom	--	6
30	full bloom	27	10
June 6	full bloom	26	10
13	full bloom	27	15
Lawrence, Kansas (first cutting)			
May 18	pre-bud	13	159
25	pre-bud	14	322
31	2/3 bud	17	315
June 6	early bloom	19	238
12	3/4 bloom	21	272
	dehydrated	--	340[1/]
Lawrence, Kansas (second cutting)			
June 26	pre-bud	6	---
July 3	pre-bud	10	10
8	bud	15	10
Lawrence, Kansas (third cutting)			
July 24	pre-bud	8	19
31	1/10 bloom	15	36
Aug. 5	1/4 bloom	21	50
12	3/4 bloom	21	51
19	3/4 bloom	21	63
26	full bloom	21	> 100
Vermillion, South Dakota			
May 23	pre-bud	11	161
31	pre-bud	14	89
June 8	bud	17	164
15	bud	24	100
23	bud	27	121
July 6	1/2 bloom	29	171
13	full bloom	30	127
19	full bloom	32	123
26	full bloom	--	148
Aug. 2	seedhead	--	151
11	seedhead	36	260
	dehydrated	--	330[1/]
Neodesha, Kansas			
May 2		11	22
9		12	76
17		14	95
23		15	81
June 1	(full bloom)	17	59
13		20	103
20		22	161

Table 3. Continued

Date (1961)	Stage of growth	Height (inches)	Coumestrol (ppm)
June 27		24	192
July 5		24	176
11		--	216
18		--	327
25		--	292
Aug. 11		30	471
		dehydrated	560[1/]
Bethlehem, Pennsylvania			
May 18	pre-bloom	--	33
25	pre-bloom	25	38
June 1	pre-bloom	26	14
8	pre-bloom	29	28
16	20% bloom	31	24
23	90% bloom	39	11
30	some seed	43	23
July 14	1/3 seedhead	44	33
25	full seedhead	--	345
Aug. 1	much secondary growth	--	256
	pelleted meal	--	380[1/]

1/ Coumestrol in final processed product.

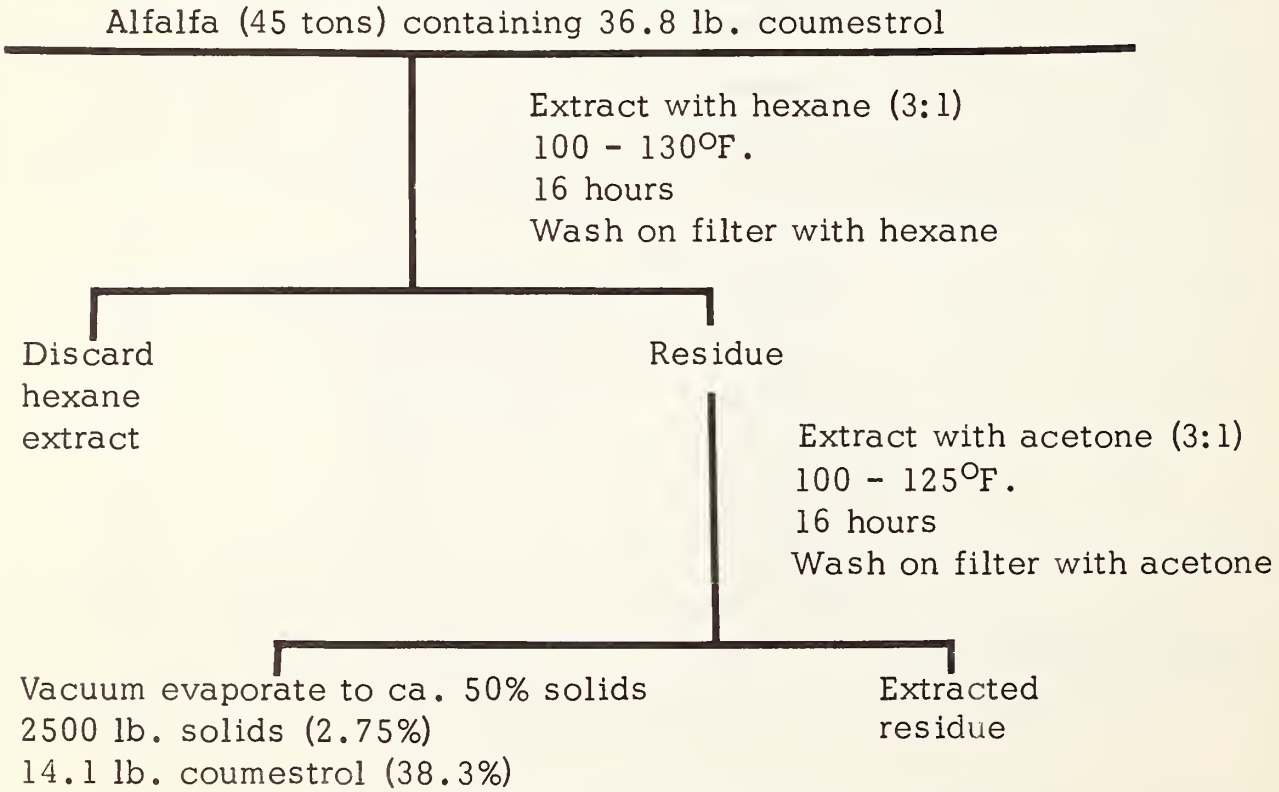

Figure 3. Preparation of a coumestrol concentrate.

This is a long-range project but one which I believe will be of greatest interest to the dehydration industry. Its object is to enable the forage processor to put out an even better product than he has been able to produce up to now.

This brings me to the end of my discussion this morning. I should like to summarize by calling your attention once again to the three ultimate objectives described before: (1) improved stability and availability of fat-soluble nutrients; (2) upgraded dehydrated products by (a) improved drying methods, (b) dry separation, and (c) wet separation; and (3) improved forage products as sources of the grass juice factor. Their accomplishment, I believe, is necessary for both solidifying the place of processed forages in present markets and in developing new and larger export markets in economically healthy countries.

GROWTH FACTORS IN DEHYDRATED ALFALFA MEAL FOR BEEF CATTLE

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Many excellent reviews and research papers have been published in the past few years pointing out the nutritional value and importance of dehydrated alfalfa meal in feedlot rations and range supplements for beef cattle. Some of the obvious and proved nutritional factors furnished by "dehy" are as follows: (1) highly digestible protein, (2) excellent source of both major and minor mineral elements, (3) superior source of unidentified growth factor(s) for the stimulation and improvement of bacterial breakdown of feeds in the rumen, and (4) abundant source of carotene (provitamin A).

This paper summarizes a recent experiment conducted at Purdue University on the effect of vitamins A and E, carotene, and dehydrated alfalfa on the performance of steers. This may help in clarifying some of the confusion in regard to the conversion of carotene to vitamin A and also present additional evidence of a factor in dehydrated alfalfa meal which improves the feed efficiency in beef cattle.

Experimental Procedure

This experiment was designed with the following objectives: (1) to determine the relative effectiveness of beta-carotene, carotene from dehydrated alfalfa meal, and vitamin A on the growth, feed efficiency, blood vitamin A values and liver storage in steers, (2) to determine the effect of feeding vitamin E singly and in combination with vitamin A, (3) to study the effect of

feeding 0.5 lb. of dehydrated alfalfa pellets (134,000 IU/lb.) on steer performance and blood and liver values.

Seventy-two steer calves averaging 525 pounds were divided equally into 12 lots of six each in a 2 x 6 factorial experiment in which one-half the lots received 0.5 lb. of dehydrated alfalfa meal pellets per steer daily. The basal ration was self-fed, and the composition is given below:

Ingredient	Percent
Rolled shelled corn	69.0
Ground corn cobs	20.0
Soybean oil meal (44%)	10.0
Bonemeal	0.7
Salt with cobalt	0.3
	100.0

The basal ration contained 10.0 percent protein, 0.25 percent calcium, 0.38 percent phosphorus, and 0.41 mg. of carotene per pound. The treatments were as follows:

No "dehy" alfalfa pellets	Vitamin supplements	0.5 lb. "dehy" alfalfa pellets per steer daily
Lot 1	Control	Lot 7
Lot 2	Vitamin A-12,500 IU	Lot 8
Lot 3	Vitamin A-25,000 IU	Lot 9
Lot 4	Beta-carotene-50,000 IU	Lot 10
Lot 5	Vitamin E-40 IU	Lot 11
Lot 6	Vitamins A & E 12,500 IU & 40 IU	Lot 12

A fresh supply of dehydrated alfalfa pellets was furnished each month (courtesy of A. R. McFadden, National Alfalfa Dehydrating and Milling Co., Kansas City, Mo.). On analysis the pellets contained 18 percent crude protein and 134,000 IU of vitamin A per pound. A dry stabilized vitamin A palmitate product containing 325,000 IU of vitamin A per gram was used as the supplemental source of biologically active vitamin A (supplied by Hoffmann-LaRoche, Inc., Nutley, N. J.). Vitamin E was an alpha-tocopherol acetate product with a potency of 125,000 IU per gram. Beta-carotene beadlets with an activity of 41,167 IU per gram were used (supplied by Hoffmann-LaRoche, Inc.).

Summary of Results

1. A detailed summary of the results is presented in Tables 1 to 9.
2. Fortifying a ground-ear-corn and soybean-meal ration with either 12,500 or 25,000 IU of vitamin A or 50,000 IU of vitamin A from beta-carotene resulted in a 23 to 32 percent increase in gain and a 10 to 20 percent improvement in feed efficiency (Tables 1 and 2).
3. Vitamin E fed at 40 IU per steer daily either singly or in combination with vitamin A did not significantly increase gain but improved feed efficiency 7 percent when fed alone (Tables 1 and 2). Blood vitamin E values were increased by either feeding vitamin E or dehydrated alfalfa meal (Table 8).

4. Feeding 0.5 lb. of dehydrated alfalfa meal per steer daily without supplemental vitamin A caused a 23 to 25 percent increase in daily gain and a 9 to 17 percent improvement in feed efficiency (Table 3).

5. Dehydrated alfalfa meal showed no significant stimulation in daily gain of steers fed vitamin A or beta-carotene, but there was some improvement in feed efficiency (Table 3).

6. There was no response to either vitamin A or carotene when steers were fed 0.5 lb. dehydrated alfalfa pellets daily furnishing 67,000 IU of vitamin A (Tables 1 and 2).

7. Feeding 0.5 lb. dehydrated alfalfa meal daily did not increase the storage of vitamin A in the liver or maintain normal vitamin A blood levels when "dehy" was fed as the only supplementary source of vitamin A. These data indicate that higher levels of "dehy" pellets are needed, about 1.0 lb. daily or more, to provide liver storage and normal blood vitamin A levels (Tables 5, 6, 7) in the absence of a vitamin A supplement.

8. On an average, feeding 0.5 lb. of dehydrated alfalfa meal per steer daily significantly increased daily gain 8 percent, improved feed efficiency 7 percent and resulted in a significant increase in vitamin A, carotene, vitamin E and cholesterol in the blood and vitamin A and carotene in the liver (Table 9).

9. Considering steer performance and vitamin A in the blood and liver, it appears from these data that 50,000 IU of vitamin A activity from beta-carotene is equivalent to 12,500 IU from preformed vitamin A palmitate.

10. From the standpoint of maximum performance of steers and blood and liver components, these data indicate that a combination of 12,500 to 25,000 IU of vitamin A plus 0.5 lb. dehydrated alfalfa should be fed daily.

Table 1. Effect of vitamins A, E, carotene, and "dehy" on gains of steers

Treatment	No "dehy"		1/2 lb. "dehy"	
	Daily gain, lbs.	Increase, %	Daily gain, lbs.	Increase, %
Control	1.71	0	2.14	0
12,500 IU vitamin A	2.11*	23	2.21	3
25,000 IU vitamin A	2.20*	29	2.21	3
50,000 IU carotene	2.26*	32	2.17	1
40 IU vitamin E	1.84	8	2.26	6
12,500 IU vitamin A + 40 IU vitamin E	2.16*	26	2.24	5

* Significant at 1% level.

Table 2. Effects of vitamins A, E, carotene, and "dehy" on feed efficiency of steers

Treatment	No "dehy"		1/2 lb. "dehy"	
	Feed/cwt. gain, lbs.	Improvement, %	Feed/cwt. gain, lbs.	Improvement, %
Control	1005	0	833	0
12,500 IU vitamin A	905	10	829	0.5
25,000 IU vitamin A	877	13	833	0
50,000 IU carotene	809	20	850	-2.0
40 IU vitamin E	939	7	853	-2.4
12,500 IU vitamin A + 40 IU vitamin E	924	8	855	-2.6

Table 3. Effect of dehydrated alfalfa meal on gain and feed efficiency

Treatment	Increase daily gain, %	Improvement feed efficiency, %
Control	25	17
12,500 IU vitamin A	5	8
25,000 IU vitamin A	0.5	5
50,000 IU carotene	4	5
40 IU vitamin E	23	9
12,500 IU vitamin A + 40 IU vitamin E	4	7
Average	8	7

Table 4. Vitamin A in blood plasma (no "dehy" alfalfa)

Treatment	Days on test		
	0	89	202
	mcg./100 ml.		
Control	43	40	10
12,500 IU vitamin A	43	74	29
25,000 IU vitamin A	43	73	36
50,000 IU carotene	41	62	23
40 IU vitamin E	50	55	11
12,500 IU vitamin A + 40 IU vitamin E	42	74	25

Table 5. Vitamin A in blood plasma (0.5 lb. "dehy" daily)

Treatment	Days on test		
	0	89	202
	mcg./100 ml.		
Control	51	63	27
12,500 IU vitamin A	40	74	37
25,000 IU vitamin A	49	81	41
50,000 IU carotene	44	72	34
40 IU vitamin E	45	55	27
12,500 IU vitamin A + 40 IU vitamin E	47	72	37

Table 6. Vitamin A in blood plasma (final values, 202 days)

Treatment	No "dehy"	1/2 lb. "dehy"
	mcg./100 ml.	
Control	10	27
12,500 IU vitamin A	29	37
25,000 IU vitamin A	36	41
50,000 IU carotene	23	34
40 IU vitamin E	11	27
12,500 IU vitamin A + 40 IU vitamin E	25	37

Table 7. Vitamin A storage in liver (228 days)

Treatment	Mcg. vitamin A/gram liver	
	No "dehy"	1/2 lb. "dehy"
Control	1.40	1.49
12,500 IU vitamin A	5.89	10.26
25,000 IU vitamin A	17.12	18.59
50,000 IU carotene	1.36	4.60
40 IU vitamin E	0.52	3.44
12,500 IU vitamin A + 40 IU vitamin E	1.57	9.46
Average	4.64	11.04

Table 8. Vitamin E in blood serum

Treatment	Mcg. vitamin E/100 ml.	
	No "dehy" 107 days	1/2 lb. "dehy" 107 days
Control	38	55
12,500 IU vitamin A	30	49
25,000 IU vitamin A	20	32
50,000 IU carotene	47	58
40 IU vitamin E	49	50
12,500 IU vitamin A + 40 IU vitamin E	43	47
Average	38	48

Table 9. Effect of dehydrated alfalfa meal

	No "dehy"	1/2 lb. "dehy"	% Change
Daily gain, lb.	2.05	2.21	8
Feed/cwt. gain, lb.	910	842	7
Blood Analysis			
Vitamin A, µg./100 ml.	22	34	55
Carotene, µg./100 ml.	72	163	126
Vitamin E, µg./100 ml.	38	48	26
Cholesterol, µg./100 ml.	110	136	24
Liver Values			
Vitamin A, µg./gm.	4.6	11.0	139
Carotene, µg./gm.	10.4	12.0	15

GROWTH FACTORS FOR SHEEP, WITH PARTICULAR REFERENCE TO ESTROGENIC MATERIALS

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For a number of years the concept of "growth factors," or substances that affect animal metabolism so as to stimulate growth, has intrigued sheep producers as it has those engaged in other types of livestock production. In general, such materials can be broadly classified as "nutrient" and "non-nutrient" in nature. Nutrient substances that stimulate growth may be further compartmentalized into known nutrients which function simply because they overcome some deficiency in the diet, and materials whose identity is not known but which might be of a nutrient nature. Poultry nutritionists have coined the term "unidentified growth factors" (UGF) for the latter.

Preliminary studies. It has become increasingly clear that some of the previously unidentified growth factors may not be listed among substances commonly thought of as nutrients, but may instead perform some other stimulatory function. One type is the antibiotics, and a considerable amount of research attests to their effects in rations for fattening lambs. Since lambs, as ruminants, depend extensively upon the symbiotic powers of their rumen flora, there was originally some hesitation in introducing known antibacterial agents into their diets. Indeed, some of the first experiences were discouraging, and listed loss of appetite, diarrhea, and loss of weight in antibiotic-treated lambs (16, 33). Later trials, involving considerably lower levels of antibiotics, began to show more promising results. Hatfield and Garrigus (22) summarized results of 3 experiments involving 190 lambs and showed that rations supplemented with 5.5 to 7.6 mg. of chlortetracycline per pound gave daily gains consistently higher (0.055 $\pm$ .014 lb.) than controls. These experiments involved a concentrate feed offered with free-choice alfalfa hay. Others showed a difference in antibiotic effect with ration composition, indicating beneficial responses to chlortetracycline with rations containing 80 percent roughage and 20 percent grain, but not at higher grain levels (19). Other investigators, however, have not confirmed this difference. In general, the antibiotic growth response with sheep has been somewhat erratic (5) and has been influenced by presence of subclinical disease as well as nutrition and other environmental factors.

More consistent growth stimulation has been achieved in sheep by hormonal materials. Effects of hormones upon growth processes have been known for some time and can be dated, indirectly at least, to studies on fowl testes carried out by Berthold in 1849 (2). Certainly common observations of differences in growth characteristics by male, female, and castrated animals, which varied in their hormone concentrations, were suggestive of hormonal-growth relationships. Research in the field of hormonal growth stimulants was at first slow and expensive, since the active substances had to be isolated from animals and purified before use with other animals. Such work became possible after the production of the first crystalline estrogen by Doisy in St. Louis in 1929 (18) and received tremendous impetus from the successful synthesis of diethylstilbestrol (DES) by British workers in 1938 (17). Availability in quantity of this latter compound stimulated both basic research into mode of action of estrogens and widespread application in domestic livestock practice.

Early experiences with estrogens in laboratory animals were not encouraging. It was shown for example that DES or other estrogenic materials accelerated ossification of the epiphyseal joints and actually reduced true growth. In fact, depression of growth was the usual occurrence following estrogen administration to rats (30, 31). Despite such adverse results, experiments in estrogen administration to lambs were carried out by several laboratories (3, 24, 29) and improved rates of gain and efficiency of feed conversion were recorded. As a matter of fact, a considerable amount of data has now been assembled (27) showing that sheep and cattle are particularly responsive to estrogenic growth-stimulation--considerably more so, for example, than are swine.

Andrews' laboratory at Purdue University, which has provided much of the experimental data on estrogen effects in domestic animals, has summarized early experiences to the effect that "there has been almost complete agreement that the subcutaneous administration of DES, dienestrol, or estradiol plus progesterone in compressed pellets has significantly improved growth rate and feed efficiency in both ewe and wether lambs" (4). The results relating to oral administration of hormones have been less consistent but generally indicative of growth improvement. Hale (21) noted varying responses to DES fed to lambs over a wide range from 2 to 600 micrograms per pound of ration, while North Dakota workers showed increased gains by lambs given 0.5 to 2.0 milligrams DES daily (25). There was an apparent increase in the extent of gains as the amount of DES was increased.

Considerable study has been devoted to the mode of action of estrogenic stimulation of growth. Some of the earlier work suggested that lamb gains resulting from DES implantation were largely due to increased tissue moisture content (35) and hence economically undesirable; however later studies with various species have shown increased nitrogen retention (ruminants : 15, 34) and stimulated protein synthesis (rat : 1). In terms more common to livestock production, administration of DES to lambs has frequently caused a lowering of carcass grade (36) presumably by hastening the growth of early-developing tissue, muscle, with the result that the animals reach acceptable market weight before they are fat. On the other hand, experiments with beef cattle have shown no detrimental effects on eating quality, as judged by tenderness scores, regardless of lower grades, with DES (14). These findings, coupled with the observation that estrogen administration has rather consistently improved the efficiency of feed conversion by fattening lambs (26, 23) have brought hormonal growth stimulation into sharp focus as a matter of practical concern to the sheep producer.

Recognition of plant estrogens. A further milestone in the evaluation of hormonal growth stimulation came with the recognition that certain plants contained materials having estrogen-like qualities, when ingested by animals. Pioneer studies in this area are usually credited to Bennetts and his associates in Australia who reported in 1946 that a local variety of subterranean clover contained some substance which interfered with reproduction in sheep (7). Identification of the substance in question as genistein (12) led to surveys of a wide range of plant species and the demonstration that estrogenic materials occurred in many of them (13). The history of these investigations has been presented in some detail by Bickoff (8, 9, 10).

Although the first implications of forage estrogens in domestic animal production were of a negative nature--being concerned principally with reproductive difficulty--it was soon realized that other effects, such as growth stimulation, which had been so frequently associated with natural or synthetic estrogens, might ensue. Bartlett (10) suggested in 1948 that the beneficial effects of spring pasture on milk production by dairy cattle might be due to estrogens in the forage. In 1957, Story and others at the Iowa Agricultural Experiment Station (32) demonstrated growth responses in lambs following the feeding of methanolic extracts from clover hay which contained 1 to 2 micrograms of DES equivalent per feeding.

Coumestrol and alfalfa. Still another significant development took place with the demonstration by scientists of the Western Regional Research Laboratory at Albany, California, of the presence of a new estrogen, coumestrol, in Ladino clover (11). Subsequent surveys showed this material to be present in alfalfa forage and in commercially prepared alfalfa meals in widely differing concentrations, and cooperative studies were set up between the Laboratory and the Oregon Agricultural Experiment Station. Briefly, these experiments involved the assay of alfalfa for estrogenic potency and the feeding of both high- and low-potency samples to fattening lambs.

The first two trials conducted in this way have been reported by Oldfield (28) and by Fox and Oldfield (20). In each of these trials, results were encouraging in that increasing levels of coumestrol activity yielded improved rates of gain, and further yielded lamb carcasses judged superior by organoleptic testing to those from low-coumestrol controls. Some question remained, however, as to the effects that other variables in the rations, other than the coumestrol content, might be exerting on the results. To reduce such incidental variables, a third trial was devised in which acetone extracts of alfalfa would be blended, in varying concentrations, with a standard basal ration. This was accomplished, and pelleted diets containing 18, 51, 114, and 151 parts per million of coumestrol were produced. The results of this trial showed once again a favorable growth response for the two higher levels of alfalfa coumestrol, as shown in Table 1.

Table 1. Lamb gains on different levels of alfalfa coumestrol

Lot No.	Ration coumestrol ppm *	Mean weight gain, lbs.
1	18	28.9 ± 2.3**
2	51	28.1 ± 2.2
3	114	33.6 ± 3.3
4	151	34.7 ± 3.2

* Coumestrol determined chemically (16) at the Western Regional Research Laboratory. ** Standard error of the mean.

In an effort to describe more fully any changes related to the dietary estrogen content, chemical analyses were run on loin muscle taken from the same area from lambs on all treatments (Table 2).

Table 2. Mean dry matter, protein, fat and ash content of lamb tissues

Lot No.	Dry matter, %	Crude protein, %	Ether extract, %	Ash, %
1	24.46	78.01	9.62	4.36
2	25.91	78.26	9.82	4.28
3	26.72	77.52	9.27	4.26
4	26.00	77.21	10.66	4.28

These data (Tables 1 and 2) suggest that an improvement in growth rate of lambs may be related to elevated levels of plant estrogen (in this case, coumestrol from alfalfa) in the ration. Moreover, they suggest that such gains are composed of useful tissue rather than moisture and are of economic value.

Differences in protein, fat, and ash content between treatments were insignificant and may be interpreted simply as increased growth of "normal" tissue. Organoleptic tests on rack roasts confirmed earlier trials in that the high-estrogen-fed groups were more desirable in tenderness and juiciness.

It would appear that level of plant estrogen fed is rather critical for sheep, and that a minimal level is necessary to elicit growth response. The response appears to reach a plateau rapidly, and additional estrogen levels no longer produce increased gains. Further studies are required to sharply define optimal levels, to chart the effects of plant estrogens in feedstuff combinations, and to investigate the stability of such compounds in storage and feed processing. Results should be directly applicable to the production of crops, like alfalfa, which are known to contain estrogenic activity.

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DARK YOLK EGGS FOR THE FOOD INDUSTRY--FACTS, FALLACIES AND FRUSTRATIONS

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I was invited to discuss this topic because I happen to be an economist interested in that area. I obtained a master's degree some 30 years ago, as a result of feeding hens various diets and checking the effects on yolk color. I try to review the literature as it comes out. But I expect the basic reason for my appearance here is the fact that the Ful-O-Pep Division of The Quaker Oats Company has been actively assisting several of our customers to produce dark-yolk-specification eggs for the past 30 months.

It is not my purpose here to tell you how we do it. The exact details are still a "trade secret." I was asked to - (1) point out the potential for you in this area, (2) to review the technical and popular literature of 1962 and attempt to bring you up to date, and (3) to point out some of the many headaches in bringing together--in a mutually profitable fashion--the knowledge of technical men, the sales of interest to you, the desires of the ultimate purchaser, and the practical know-how.

Carlson et al. (1962) put it very well - "Much interest has been encountered in the enhanced pigmentation of egg yolks. Hens kept in confinement for production of eggs for table-egg markets are for the most part routinely fed diets that cause relatively light-colored yolks. Bakers and noodle manufacturers in particular are paying premiums for darker yolks. So it has been necessary for egg processors to obtain dark-yolked breaking stock from controlled production sources."

Your basic interest, of course, is in alfalfa as a pigmenter--as well as an excellent source of vitamin A and vegetable protein. What is your potential market in this area of dark-yolk production? Because of emphasis on convenience foods and "built-in maid service" U. S. consumption of processed eggs is rising. It amounts to some 7 to 10 percent of all eggs. If 5 percent of this were diverted to dark-egg production and only 15 percent "dehy" was used, this segment alone would use over 100,000 tons per year! But this must be a special product--lower in fiber, higher in protein, and with a stiff guarantee as to units of vitamin A or milligrams of xanthophylls per pound of product.

In the January 12, 1963, issue of Feedstuffs, a member of your industry has a most interesting ad--selling the basic idea of 20 percent "dehy" as a pigmenter. This is good. You do have competition. But the point I will make later is that there is more to the production of dark yolk eggs than just having the assumed requirement of xanthophylls in your ingredient.

We mentioned competition. Carlson et al. compared alfalfa, grass and clover meal, algae meal, paprika and xanthophyll concentrates. Perdue (1962) reported on the effectiveness of a protein-rich algae product. Fritz (1962) investigated Mexican marigold flower petal meal--among others--that is extremely high in xanthophyll (4500 mg./lb.). Bauernfeind (1962) discussed the work of his company with Beta-Apo-8^1-Carotenal and Canthaxanthin, as pigmenters, under laboratory conditions. Couch (1962) reported on work at Texas with pigmentation. He said that "High grade dehydrated alfalfa meal offers the cheapest source of xanthophylls among natural ingredients, including corn and corn gluten meal. This product is still high in fiber and low in energy in comparison to the other two mentioned .... The price the feed man has to pay for products cleared by FDA will determine use of some products. It has been shown with a number of micro nutrients that as usage increased, price decreased. Such could very well be the case in pigmenters."

The basic information required for the production of dark yolk eggs has been known for at least 25 years. The darkest yolk can be produced by hens running out-of-doors in the spring of the year on rye pasture. But today's poultryman cannot live with that type of production. Nor can egg processors afford the costs of short-term plant operations or tying up of capital "in the box" freezer.

Some of the basic problems are - (1) instability of vitamin A, or xanthophylls, (2) variation of apparent ability of hens to utilize all sources of xanthophyll equally well, (3) increased feed costs per unit of color, (4) differences in breeds and strains in their ability to utilize color, as well as total yolk yield (Irwin, 1963), (5) effect of high alfalfa (fiber?) on feed efficiency and length of lay, and (6) the effects of the combination of high temperatures and age on such things as dehydrated alfalfa, manufactured feed, laying hen, egg, and color of frozen egg.

The frustrations in this problem are many. The manufacturers of noodles and cakes want a darker yolk. It costs more to produce a Nepa 5 (dark yolk) than it does the normal Nepa 2-3 (table egg). The breakers are not sure they can pay the added cost of 1 cent per dozen eggs.

Many attempts have been made to apply the results of the laboratory to commercial field production. In most cases when adequate amounts of 20 percent protein "dehy" have been used (25 mg. of xanthophyll--Couch, Carlson--or 15 to 20 percent of 20 percent protein "dehy"--Couch), production slumps, or birds even go out of production, and pounds of feed per dozen eggs increases. Fritz (1962) points out the necessity of antioxidants for stability of xanthophyll but states that "Other factors, too, must be considered, such as health of birds, and more xanthophyll is needed for yolk pigmentation in hot weather."

This leads into considerations of meeting these more exacting specifications of producing a given Nepa in hot weather. The same weather during which hens under pre-1940 conditions produced darker yolks, proved to be a time of difficulty in the 1960's. As Fritz pointed out, the feed needs a higher xanthophyll content for the same pigmentation in hot weather. There is also some evidence that the mixed feeds lose their xanthophyll faster in hot weather. The industry generally needs more knowledge--not on how to produce a dark yolk but specifically on what affects xanthophyll retention in feed ingredients, influences the utilization of it by the hen, and in the egg--both shell and frozen--what inhibits the loss of color.

Perhaps answers to these "specifics" would help the egg products industry to answer the question - Can we afford to pay more for dark yolk eggs on a year-round basis? It would certainly help the egg production industry--now in a great state of flux--to decide its own answers to the question, Can we afford to produce dark-yolk eggs? Dark-yolk specification eggs are now being produced profitably on a limited basis. It is not sufficient simply to buy your high-protein, high-guaranteed potency of either vitamin A or xanthophylls. It now requires a lot of programming and exacting controls. More answers from research could broaden the market for your product because--so far--dehydrated alfalfa is the cheapest source of pigment for dark-yolk eggs.

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VITAMIN A ACTIVITY OF ALFALFA IN CHICKENS

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Vitamin A is one of our oldest known vitamins, but many years of painstaking research were required before the chemistry of vitamin A and carotene were unraveled and their synthesis achieved. Despite these major accomplishments, we still have much to learn about the role of these essentials in health and disease. This is explained by their complex chemical structures, and by the fact that over a dozen different biologically active forms of vitamin A and natural carotenoids are now known.

Review of Literature

As early as 1919 it was suspected that carotene, found in leaves and other natural products, was vitamin A active (Steenbock, 1919); but shortly doubts arose (Steenbock *et al.*, 1920). By 1925 it had been established that alfalfa was a rich source of vitamin A activity, and that activity seemed to be associated with carotene. In 1928 it was demonstrated that vitamin A activity of as little as 0.005 mg. of carotene per day supported good growth of rats (Euler *et al.*, 1928). In 1929 it was proved that carotene was converted to vitamin A in the animal body by feeding carotene to vitamin A depleted rats and finding appreciable quantities of vitamin A deposited in the livers (Moore, 1930). It is of interest that this development came 98 years after a chemist first isolated carotene from plant tissue without realizing its nutritional value.

A study reported in 1923, in which an alfalfa extract was used as a source of fat soluble A (vitamin A), was probably the first to demonstrate clearly that chickens required this nutritional factor (Emmett and Peacock, 1923). By the early 1930's, dried or dehydrated alfalfa had been used successfully as a vitamin A source in several poultry investigations.

Carotene is a valuable and proved source of vitamin A for chickens. The National Research Council's Committee on Poultry Nutrition recognizes that equal numbers of units of vitamin A activity from carotene and from true vitamin A are equivalent as vitamin A sources for growing chickens (N.R.C. 1960), but that fact sometimes is questioned or even forgotten. In the past some confusion has been reported over the conversion ratio of carotene to vitamin A when dehydrated alfalfa is used. The generally accepted conversion for poultry and rats is on the basis of 0.6 microgram of beta-carotene being equivalent to 1.0 IU of vitamin A (Rutter, 1961).

The stability of carotene in dehydrated alfalfa meal is improved appreciably by the addition of four or five percent of an animal fat or a vegetable oil to the meal (Mitchell et al., 1954; Mitchell and Silker, 1955; Van Atta et al., 1953). Parrish and Mitchell (1958) conducted chick-growth experiments to compare the biological availability of the carotene of stabilized and unstabilized alfalfa meals. They found that use of oil, antioxidant, and heat to improve stability of carotene in alfalfa meal, as measured by photometric means, does not adversely affect the utilization of the carotene by chicks.

Recent reports (Camp et al., 1955; Ely, 1959; Gledhill and Smith, 1955; Olsen et al., 1959; Williams, 1962) have indicated that carotene is not utilized efficiently by poultry as a vitamin A source. Some doubt that natural carotene carriers are satisfactory sources of vitamin A activity for poultry. Earlier literature (Wilson et al., 1936; Record et al., 1937; Petering et al., 1941; Kramke et al., 1952) supports the equivalence of units of activity from vitamin A and beta-carotene for growth of poultry.

KSU growth studies. In our laboratory newly hatched chicks were depleted of vitamin A reserves for 6 to 14 days and were fed eight weeks to test utilization of vitamin A activities of several samples of alfalfa meal as compared to that of USP and ANRC vitamin A standards. In four tests, comparisons were made at both 400 and 800 units of vitamin A activity per pound, and in one test at 1200 units per pound. Fresh feeds containing the required quantity of vitamin A activity were prepared weekly. In three of four tests using 400 and 800 units per pound, weight gains and liver vitamin A contents of chicks receiving the two sources did not differ significantly. Blood serum vitamin A levels, however, were significantly higher for chicks receiving ANRC vitamin A in three of the four tests. At the 1200 unit level there was not a significant difference in gain or in liver and blood serum vitamin A levels. There were no significant differences in feed conversion ratios in any of the tests. As a whole, results were as satisfactory when vitamin A was supplied by alfalfa meal as when supplied by standard vitamin A. Our results indicate that carotene as supplied by alfalfa meal is an entirely satisfactory source of vitamin A for growing chickens. Sometimes the carotene of alfalfa meal is not considered in formula calculations when alfalfa meal is added to supply other nutrients, such as protein, minerals, unknown factors, pigmenters, etc. Our data indicate that regardless of quality of meal used, full credit should be given for the carotene added when making formula calculations.

The cost of a formula feed can be lowered without reducing quality if proper account is taken of quantities of all nutrients in the alfalfa used,

Table 1. Diets for Experiments VI and VII, KSU

Component	Expt. VI					Expt. VII	
	Depletion	8% fat basal	0% fat	4% fat	8% fat	Depletion	Expt'l
	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.	lbs.
White corn	30.0	30.0	30.0	30.0	30.0	41.5	40.0
Sorghum grain	31.5	22.0	31.5	27.0	22.0	20.0	18.5
SBOM (44%)	29.0	30.5	29.0	29.5	30.5	29.0	28.0
Brewer's dry yeast	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Skim milk	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5
$CaCO_3$	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Steamed bone meal	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Stabilized lard		8.0		4.0	8.0		2.0
Crude casein							2.0
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Wesson oil	300.0	300.0	300.0	300.0	300.0	300.0	300.0
Mineral pre-mix	138.5	138.5	138.5	138.5	138.5	138.5	138.5
Vitamin pre-mix	469.5	469.5	469.5	469.5	469.5	469.5	469.5
			units	units	units		units
Vit. A[a]			b	b	b		d
Vit. A[a]			700[c]	700[c]	700[c]		d
Vit. A[a]			e	e	e		d,f

[a] Each diet contained in addition 35 to 50 units vitamin A from sorghum grain and/or lard.

[b] Each diet was fed to 4 lots receiving, respectively, 400, 700, 1000 and 1500 units vitamin A from alfalfa meal. See Table 2.

[c] Diet fed to 1 lot, source of vitamin A was ANRC standard. See Table 2.

[d] 1500 and 3000 units vitamin A given to lots receiving alfalfa meal as the source and to lots receiving ANRC standard. See Table 4.

[e] 800 units from alfalfa, and 700 units from ANRC vitamin A standard. See Table 2.

[f] Diet fed to 1 lot, half from alfalfa and half from ANRC standard. See Table 4.

Table 2. Results Experiment VI[a], KSU

Dietary fat added		Level and source vitamin A activity/lb.					
		Alfalfa meal				ANRC std.	ANRC & alfalfa
		400 units	700 units	1000 units	1500 units	700 units	1500[b] units
None	Wt. gain, gm.	820	820	813	821	875	885
	Vit. A, units/liver, av.	11	35	71	452	77	355
	Vit. A, units/100 ml. serum, av.	66	89	111	271	164	267
4% fat	Wt. gain, gm.	800	787	892	884	849	872
	Vit. A, units/liver, av.	18	46	161	618	84	864
	Vit. A, units/100 ml. serum, av.	95	118	239	350	170	324
8% fat	Wt. gain, gm.	807	888	861	914	816	915
	Vit. A, units/liver, av.	22	77	165	532	92	900
	Vit. A, units/100 ml. serum, av.	79	164	217	290	112	327

Analysis of variance:[c]

	Gains	Liver vit. A	Serum vit. A
Level vitamin A	**	**	**
Level fat	**	n.s.	**
Vit. A X fat	*	n.s.	n.s.

Multiple range test:[d]

	Level vitamin A			
Gain	400	700	1000	1500
Liver vit. A	400	700	1000	1500
Serum vit. A	400	700	1000	1500
	Level fat			
Gain	0	4%	8%	
Serum vit. A	0	4%	8%	
	Level fat X vitamin A			
Gain (4% fat)	700	400	1500	1000
Gain (8% fat)	400	1000	700	1500

[a] Twelve chicks/lot; 4 randomly selected samples used for liver and serum analysis.

[b] 700 units from ANRC standard, 800 from alfalfa meal.

[c] ** Significant at 1%; * significant at 5% level.

[d] Diets placed in order of increasing response, left to right. Those underlined by same line do not differ significantly.

Table 3. Feed consumption and feed conversion ratios. -- Experiment VI, KSU

Dietary fat added		Level and source vitamin A activity[a]					
		Alfalfa meal				ANRC std.	ANRC & alfalfa
		400 units	700 units	1000 units	1500 units	700 units	1500 units
None	Feed consumption[b]	5.72	6.07	5.63	5.52	6.08	5.90
	Feed conversion ratio	3.16	3.36	3.15	3.06	3.16	3.03
4% fat	Feed consumption	5.54	5.57	5.79	5.45	5.73	5.18
	Feed conversion ratio	3.15	3.21	2.94	2.79	2.99	2.76
8% fat	Feed consumption	5.42	5.40	5.11	5.52	5.52	5.68
	Feed conversion ratio	3.06	2.79	2.69	2.74	3.06	2.82

[a] Same activities, sources, etc. as in Table 2.
[b] Pounds, average per bird.

Table 4. Results of Experiment VII[a], KSU

	Vit. A units/lb. feed	Source of vitamin A		
		Alfalfa	Alfalfa and[b] ANRC std.	ANRC std.
Wt. gain, gm.	1500	868	873	845
Liver vit. A, units/liver[c]		381	358	571
Serum vit. A, units/100 ml.		278	259	286
Feed conversion factor		2.97	2.83	3.10
Wt. gain, gm.	3000	853	878	880
Liver vit. A, units/liver		1949	2788	3935
Serum vit. A, units/100 ml.		324	341	364
Feed conversion factor		2.86	2.99	2.88

Analysis of variance:

	Level	Source	Interaction
Gains	n.s.	n.s.	n.s.
Serum vit. A	**[d]	n.s.	n.s.
Liver vit. A	**	**	**

[a] Male Hy-line chicks, 10/lot, put on experiment following 14-day depletion period.
[b] Half the vitamin A from each source.
[c] Liver and blood serum determinations on 5 random samples/lot.
[d] ** Significant at 1%; * significant at 5% level.

including carotene. If as little as two to three percent of 100,000-unit alfalfa meal is used, 2000 to 3000 units of vitamin A are added per pound of feed.

Broiler strain chicks, depleted of their initial body vitamin A stores, have gained three pounds or more between the second and ninth weeks with a feed conversion ratio of 2.1:1, using carotene in alfalfa meal as the sole source of vitamin A (Shellenberger et al., 1960).

Using vitamin A depleted egg-strain (Hy-line) males and meat-strain (Cornish x White Rock) chicks and a high energy feed (24 percent protein, 1,060 Calories productive energy), Shellenberger et al., (1960) studied the effect of different levels of vitamin A (as carotene of alfalfa meal) in the diet on utilization of feed. Vitamin A fed as carotene in alfalfa meal at 600 units per pound or more permitted good growth.

Some of our results were obtained with vitamin A depleted chicks using even less vitamin A than listed as the NRC requirements (NRC, 1960). The findings are in harmony with most published nutritional data from the past 40 years of vitamin A research and refute some recent reports that carotene is poorly used and of limited value as a vitamin A source for chicks.

Studies from our laboratories reveal only a small storage of vitamin A in livers and blood was obtained until more than 700 to 800 units of vitamin A per pound of feed were fed. However, there is no real proof that substantial vitamin A storage is needed for optimum growth by chickens obtaining constant vitamin A intakes as they do in modern types of feeds.

Several earlier studies have shown that young chicks could utilize carotene as a source of vitamin A by the end of the first week, although one investigator stated it was ineffective unless chicks were three weeks old. Work conducted at Kansas State University (Harvey et al., 1955) revealed that chicks fed dehydrated alfalfa, when less than 12 hours old, converted carotene to vitamin A during the first day of life; and that it supported physiological functions involving vitamin A by at least the fifth day and probably earlier. Parrish and Sanford (1961) observed that provitamin A of low quality alfalfa meal is utilized effectively, but not so consistently as well as from high quality alfalfa meal.

KSU egg production studies. Carotene in alfalfa meal has been used successfully with layers over extended periods. Laying pullets have been grown and have gone through a full year of egg production using only carotene as the source of vitamin A (Shellenberger et al., 1957). Leghorn pullets were fed diets containing 2,000 units of vitamin A per pound as alfalfa meal starting at 18 weeks of age. Feeds were prepared at approximately monthly intervals. Egg production on a hen-day basis for seven months was 65 percent, with no antioxidant or other protective measures used. After four month's production, eggs were collected to check reproduction. Hatchability of 90 percent was obtained, and all chicks were normal. Another study (Shellenberger et al., 1960) was unique in that newly-hatched chicks, depleted for one week, received only carotene in alfalfa meal as the vitamin A source throughout the period of growth plus one full year of egg production. These results indicate that Leghorns can do well on carotene of alfalfa as the sole source of vitamin A through the

periods of growth and a full year of production even when feeds are not entirely consumed until one month after mixing.

Parrish and Sanford (1960) reported hens fed a vitamin A deficient basal ration supplemented with 3000 units of vitamin A per pound of feed supplied by alfalfa meal averaged 4 percent higher production than those supplemented with 1500 units of vitamin A. Parrish and Sanford (1960a) found that increasing levels of furazolidone (feed additive used for disease control) appeared to decrease egg weight, improve feed conversion ratios, and increase egg production slightly.

Zimmerman et al. (1961) conducted a study to determine the value of carotene of alfalfa as the source of vitamin A activity and to determine the effect of furazolidone on the utilization of carotene of alfalfa by laying hens. A significantly higher rate of egg production was noted when 3000 units of vitamin A activity per pound of feed supplied as carotene of alfalfa was fed as compared to 1500 units. From the standpoint of egg production, mortality and feed conversion, it can be concluded that laying hens can utilize carotene from alfalfa as their sole source of vitamin A. (Egg production on a hen-day basis was from 65 to 77 percent by the various groups of hens.)

Parrish and Sanford (1962) studied the relative value of the vitamin A activity of carotene of alfalfa meal and of stabilized vitamin A during the laying period. In all experiments, high quality alfalfa meals, vitamin A activities between 270 to 580 units per gram were used. Meals were stored under sharp freeze conditions. Quantities added to diets were based on monthly carotene determinations. Diets were mixed each week. Alfalfa was removed from the freezer just prior to mixing. Inbred-crossbred egg-strain pullets were fed the basal diet (Table 5) plus 2000 or 3000 units of vitamin A per pound as carotene of alfalfa or 2000 units as stabilized vitamin A (Stabimix). Egg production data were collected daily and calculated by 28-day periods. Average egg production was higher when stabilized vitamin A was fed, but production from all three groups was good. Feed conversion was best by the pullets fed the ration supplemented with stabilized vitamin A. There was no significant differences in egg weights, shell weights, percent shell or Haugh unit scores.

Parrish and Sanford (1962) studied the effect of feeding furazolidone on relative utilizations of provitamin A of alfalfa meal and of stabilized vitamin A as vitamin A sources for caged layers. Inbred-crossbred pullets were fed the vitamin A deficient ration supplemented with 3000 units vitamin A per pound of feed either as stabilized vitamin A or alfalfa meal as shown in Table 7. Egg production for all lots was between 64.9 and 74.0 percent. Differences between lots were highly significant. Increasing levels of the drug caused a decrease in egg weight, shell weight and yolk weight. Samples were obtained at the end of the experiment to check effect of treatment on vitamin A content of eggs and storage in the livers. Nonsignificant differences in amounts of vitamin A per egg yolk were found, whereas, the livers of birds getting alfalfa meal contained between 96 and 231 mmg. per liver; and livers of birds getting stabilized vitamin A had 3463 and 4277 mmg. per liver. This was a highly significant increase over those getting alfalfa. The level of drug did not appear to have a significant effect on liver or egg vitamin A contents.

Table 5. Composition of KSU basal diet - laying pullets 1961-62

Ingredients	
Sorghum grain, ground	73.5 lbs.
Wheat standard middlings	4.0 "
Soybean oil meal (44% solv. extr.)	13.0 "
Fish meal (Menhaden)	1.5 "
Brewer's dried yeast	1.5 "
Salt (NaCl)[1]	0.5 "
Ground limestone (calcium carbonate)[1]	4.0 "
Steamed bone meal[1]	2.0 "
Total	100.0 lbs.
	Add/100 lbs.
$MnSO_4$[1]	23 gms.
Vitamin K (Klotogen F; 8 gm./lb.)[2]	4.8 "
D-L Methionine[2]	46 "
Vitamin D_3 (15,000 ICU/gm.)[2]	5 "
Proferm$_{12}$ Vitamin B_{12})[2]	19 "
Merck 58-A(R) (Vitamin pre-mix)[2,3]	23 "
Choline chloride (25% mix)[2]	88 "

[1] Mineral pre-mix.

[2] Additive pre-mix.

[3] Vitamin pre-mix contains, per lb. 2 g. riboflavin; 2.68 g. pantothenic acid; 6 g. niacin and 20 g. choline chloride.

(R) = Registered trade-mark.

Table 6. Percent production for floor pen layers by 28-day periods, December, 1961 to October, 1962, KSU
(Hen-day basis)

Vitamin A level/lb. and source	I 2000 units alfalfa	II 2000 units stabilized A	III 3000 units alfalfa
Period[a]		% production	
1	75.0	74.2	68.0
2	68.3	72.5	64.3
3	76.3	73.9	71.9
4	78.7	75.5	75.4
5	78.9	82.1	78.1
6	77.0	86.7	75.5
7	66.1	80.1	71.3
8	66.7	77.8	69.8
9	63.5	71.6	68.5
10	61.1	64.3	64.7
11	58.7	65.9	58.7
av.	70.0	75.0**	69.7
Feed conversion, lbs./doz. eggs	4.19	4.04	4.07

Analysis of variance --- percent production;

Treatments	**
Periods	**
Egg weight	n.s.
Shell weight	n.s.
Percent shell	n.s.
Haugh units	n.s.

[a] 28 days each, December to October. During test, 2 birds lost in lot I, 3 in lot II, and 0 in lot III.

** Production highly significantly greater than by lots I and III.

Table 7. Production data on caged layers,[a] KSU

Lot	Source vit. A	Furazolidone[b] addition	%[c,d] prod.	Feed conversion ratio[e]	Egg[d] wt., gm.	Shell[d] wt., gm.	Yolk[d] wt., gm.	%[d] yolk	%[d] shell	Haugh units
1	Alfalfa	0	74.0	3.5	60.7	5.06	17.9	29.5	8.4	74.3
2	Alfalfa	1/2	66.9	3.7	59.0	5.01	17.5	29.6	8.5	77.3
3	Alfalfa	1	73.4	3.7	57.8	4.76	17.2	29.7	8.3	76.0
4	Alfalfa	2	65.9	3.8	57.5	4.70	16.7	29.1	8.2	76.0
5	Stabilized A	0	64.9	4.1	61.8	5.23	18.0	28.9	8.5	74.3
6	Stabilized A	2	74.0	3.6	58.2	4.81	17.0	29.2	8.3	74.2

[a] Data collected in 18 periods of 14 days each. One bird lost in each of lots 2, 3 and 4, apparently from visceral leucosis. All lots received 3000 units vitamin A per lb. feed.

[b] Given as nf-180(R) (in lbs./ton).

[c] Hen-day basis.

[d] Analysis of variance revealed effect of treatment on each measurement made was highly significant. Feed conversion ratio not analyzed statistically.

[e] Pounds of feed per dozen eggs.

(R) = Registered trade-mark.

Table 8. Vitamin A data on caged layers,[a] KSU

Lot	Source vit. A	Furazolidone[b] addition	Egg yolk				Liver			
			Carotenoids vitamin A				Carotenoids vitamin A			
			mmg./ gm.	mmg./ yolk	mmg./ gm.	mmg./ yolk[c]	mmg./ gm.	mmg./ liver	mmg./ gm.	mmg./ liver[c]
1	Alfalfa	0	10.4	206	9.8	196	8.8	235	5.8	156
2	Alfalfa	1/2	8.7	162	9.2	173	10.3	340	6.4	231
3	Alfalfa	1	10.0	165	10.1	165	6.8	250	2.8	96
4	Alfalfa	2	8.8	152	10.9	189	11.3	325	7.1	208
5	Stabilized A	0	1.04	19.6	9.3	173	2.0	66	103	3,463
6	Stabilized A	2	1.05	19.1	9.7	176	1.5	46	132	4,277

[a] Data from 4 birds of each lot at end of experiment.

[b] Given as nf-180(R) (in lbs./ton).

[c] Vitamin A data per yolk and per liver treated by analysis of variance. Yolk vitamin A differences n.s. Liver vitamin A of lots getting stabilized vitamin A were highly significantly different than those getting alfalfa. Differences among groups getting alfalfa were not significant.

(R) = Registered trade-mark.

Table 9. Results on 8-month test with floor pen laying hens, KSU

Lot	Treatment	Egg production %[1]	Feed Conversion ratio[2]	Mortality (%)[3]	Egg carotenoids[4] content mmg./yolk	Egg vit. A[4] content units/yolk
1	1500 units vit. A	68	4.62	4	12	97
2	1500 units vit. A and 1/2#/ton nf-180(R)	65	4.40	2	13	110
3	1500 units vit. A and 1#/ton nf-180	66	4.37	4	11	75
4	1500 units vit. A and 5#/ton nf-180	70	3.91	0	8	99
5	3000 units vit. A	67	4.40	4	11	130
6	3000 units vit. A and 1/2#/ton nf-180	72	4.24	6	17	142
7	3000 units vit. A and 1#/ton nf-180	72	4.08	4	18	162
8	3000 units vit. A and 5#/ton nf-180	72	3.91	6	19	201

[1] Hen-day basis.
[2] Feed per dozen eggs.
[3] Adjusted for a few accidental losses.
[4] Three eggs per group analyzed at end of experiment.
[5] Vitamin A supplied by carotene in high quality alfalfa meal.
(R) = Registered trade-mark.

Practical experience over many years has added solid support to use of alfalfa as a vitamin A source. High quality alfalfa meal is a valuable feedstuff. It has high vitamin A activity, as well as an abundance of many other nutrients (Parrish and Sanford, 1960).

Summary

Several studies have been conducted with growing chickens and with laying pullets on utilization of vitamin A as supplied by carotene of alfalfa meal. In some of our work comparisons were made with utilization from stabilized vitamin A products, and also studies were made of effects of certain drugs and types of diets on utilization. Both growing chickens and laying pullets have performed well when the vitamin A source in the diet was alfalfa meal.

A summarization of all our data indicates that chickens grew similarly when given vitamin A as either alfalfa meal or stabilized vitamin A. At higher levels of vitamin A intake, liver vitamin A storage was larger when the latter vitamin A source was used. As judged by liver and blood serum accumulations of vitamin A, chicks were found to utilize carotene of alfalfa meal efficiently as a vitamin A source in diets which also contain true vitamin A. There are indications that when the diet contains added fat, growing chickens do better if at least 1000 units of vitamin A per pound of diet are fed.

Utilization must not be confused with stability. Some earlier studies of utilization of vitamin A sources are difficult to interpret because of stability factors. Furazolidone did not significantly affect utilization of provitamin A of alfalfa meal for growth, nor for liver and blood vitamin A storage. Use of 0.02 percent ethoxyquin did not improve gain on vitamin A storage significantly. However, the higher level of ethoxyquin (0.10 percent) improved gain, but not blood and liver vitamin A storage.

Pullets kept in floor pens under natural environmental conditions performed somewhat better during a 10-month laying period when the source of vitamin A was stabilized vitamin A. More vitamin A was stored in the livers of pullets, but not in eggs, when stabilized vitamin A was compared to alfalfa meal as a source of vitamin A at higher levels (3000 units/lb. feed). Since it has not been clearly established just how high liver vitamin A storage should be, or the benefit derived from high liver storage by birds fed balanced diets, it is difficult to interpret the significance of liver storage data.

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NEBRASKA PROJECT PROGRESS REPORT

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This investigation is known as the Nebraska project because it is cooperatively supported by funds from the Department of Agriculture and Inspection of the State of Nebraska and the Western Regional Research Laboratory. The purpose is to obtain improved alfalfa products for use in commercial feeds and in feeding and to find commercial uses for whatever fractions of lower grade may be produced in achieving the first objective.

Improved alfalfa products may be defined as fractions or concentrates of alfalfa which are high in protein, vitamins, growth factors, pigments and other nutrients but low in deleterious components or those with poor digestibility characteristics. Lower-grade products might be defined as those high in fibrous constituents, low in protein and other nutrients and perhaps higher than usual in concentration of undesirable constituents such as saponins.

This is a progress report and most conclusions remain tentative. We first indicate the studies made of the various parts of the alfalfa plant; second, discuss some of the methods of separation and classification attempted with a few of the results; and, third, discuss the examination of a few commercial samples.

In order effectively to pursue an investigation to meet the objectives, it was first necessary to identify fine particles of dried alfalfa on the basis of the morphologically distinct parts of the anatomy of the plant. To do this, microscopic examinations were made and staining techniques developed for distinguishing those particles which were predominantly protein from those composed principally of cellulose or fiber. Photographic techniques were developed for permanently recording microscopic data. Many samples were studied.

Fine grinding equipment developed by the flour milling industry and called "turbomills" seems to offer some possibilities for comminution of alfalfa particles to a point where air classification of the ground material might result in high and low protein fractions. A number of samples of alfalfa from commercial plants, both dehydrated and sun-cured, were ground in a turbomill. Some of these were screened prior to milling and fractions of different mesh size were subjected to milling and air classification. Proximate analyses were made on many screen fractions, air-classified fractions, as well as on original samples.

The products of turbomilling and air classification were subjected to microscopic examination and to analysis for particle size distribution. These particle size analyses were made by a sedimentation-rate method under gravity and centrifugation. The liquid medium used is benzene. It is necessary to devote considerable time to calibration of the air classification equipment and to developing skill in the techniques of determining particle size distribution.

Although some differential has been obtained, it is evident that there is still considerable fiber in each fraction. Experience thus far indicates it would be advisable to make a coarse classification of leaf and stem and then subject the leaf portion to turbomilling and air classification.

Another problem was disclosed or rather emphasized as a result of turbomilling. Wear on the moving parts and on the interior of the housing of the turbomill was excessive as a result of grinding alfalfa of high grit content. This eventually resulted in dismantling the mill and ordering the rotor to be rebuilt, if possible of more wear-resistant materials. This circumstance halted these phases of the investigation temporarily. It is now felt that research must be undertaken to develop practical means for removal of the bulk of the grit from any alfalfa which is to be turbomilled.

As a result of this experience we are now routinely including ash determinations in the analyses of alfalfa products and in many instances, also determining grit as distinguished from total ash.

In making many sieve tests or screen analyses of alfalfa products it has become well established that the preponderance of the grit lies in the very fine fractions--finer than 100 mesh or 149 microns. We are now investigating several possible ways of removing the grit without, at the same time, losing too much of the valuable leaf material with which it is associated. As an illustration, some dehydrated alfalfa, chopped whole plant, was screened through a Sweco Separator dressed with 12, 24, 50 and 100 mesh screens. An analysis of the feed indicated 7.44 percent grit and was exceptionally high in ash (see Table 1). We find that almost half of the grit is concentrated in only 9.4 percent of the total sample.

Table 1. Illustration of grit distribution in an alfalfa sample

		% Weight	% Ash	% Grit	% Protein
	Plus 12 mesh	37.6	9.4	0.50	11.94
Minus 12	" 24 "	28.9	13.0	2.63	22.81
" 24	" 50 "	24.1	20.5	9.63	23.88
" 50	" 100 "	1.1	50.1	24.00	16.50
" 100	"	8.3	46.3	33.85	15.63
Weighted average		--	16.6	6.34	18.31
All minus 50 mesh		9.4	46.7	32.69	15.73

Assuming we could remove the grit completely from the minus 50 mesh fraction, we would discard about 61 pounds per ton. At, say, $60 per ton this would represent a loss of about $1.83 per ton. However, in doing this we would upgrade the protein of the remainder of <u>that</u> fraction from 15.73 to 23.36 percent. The increased value of the remainder would more than offset the monetary loss of throwing away the grit. There would be some cost involved in removing the grit, but it is our opinion that the possible savings in machinery wear through abrasion would more than offset any such operating costs.

Turning now to the whole growing plant our efforts have been to determine leaf-stem ratios and physical location of the protein fiber and ash as well.

While much work has been reported in the literature regarding the leaf-stem ratio in growing alfalfa and the distribution of protein, fiber, and ash in the various fractions of the plant, it was felt that further investigation in a meticulous manner should be done on varieties with which we will be working and new varieties introduced in recent years.

The first effort in this direction was the very careful separation of freshly cut Lahontan pre-bud alfalfa plants from plots adjacent to the Laboratory. Six fractions were made and we found that the true leaf and leaf buds ran better than 40 percent protein. The leaf alone showed a fiber content of 7.6 percent while the stem ran 43.8 percent.

A more realistic approach is to combine leaf, leaf buds and midribs into one fraction and stem, skin, and petioles into a second fraction. In doing this we found that the first fraction amounted to 51 percent of the total and averaged 39.37 percent protein and 8.73 percent fiber while the second combination represented 49 percent of the dry weight and analyzed 15.9 percent protein and 37.9 percent fiber. This shows a very good increase in protein content for the high grade fraction but an even better reduction of fiber.

These results apply to only one variety at only one stage of growth. Knowing that changes do take place in leaf-stem ratio and distribution of protein, fiber, ash, etc., as the plant matures, we pursued another course by sampling a nearby field of California Common alfalfa at weekly intervals for seven weeks. Again, the plants were separated by hand, but into four parts--leaf, buds (both leaf buds and flower buds), petioles and stems. Stipules were included with petioles.

During this particular study three of the weekly cuttings were paralleled by a different technique in separation. Plants were stripped by hand using a heavy rubber glove. The remaining stems were severed as in the other procedure and the two fractions thus obtained were dried and analyzed. In general, the differences between the two methods were small enough to indicate to us that field stripping might be an excellent method of obtaining a relatively high quality of product for dehydration. This would result in a stem fraction high enough in fiber and low enough in protein that it would hardly warrant the expense of dehydration. It might better be cut with a stem crushing mower and allowed to sun cure. A good grade of sun-cured stem meal could be made which, in some respects, would be superior to much of the prairie hay now produced. Our work has thus pointed to a need for a radically different type of field operation based on development of a new leaf harvester.

Having concluded this work on California Common grown locally, it was considered advisable to obtain similar data on alfalfa of some commonly grown variety from the midwestern part of the country. To do this we made arrangements with a dehydrating plant in Kansas to clip fresh alfalfa from a field of the variety Buffalo, dry it in an oven and ship intact to our Laboratory. This was done each week for eight consecutive weeks and the same separations made as on the California Common.

Some of the results of this investigation of Buffalo are shown in Figures 1, 2, 3, 4, and 5, and Tables 2 and 3. Large samples of dehydrated

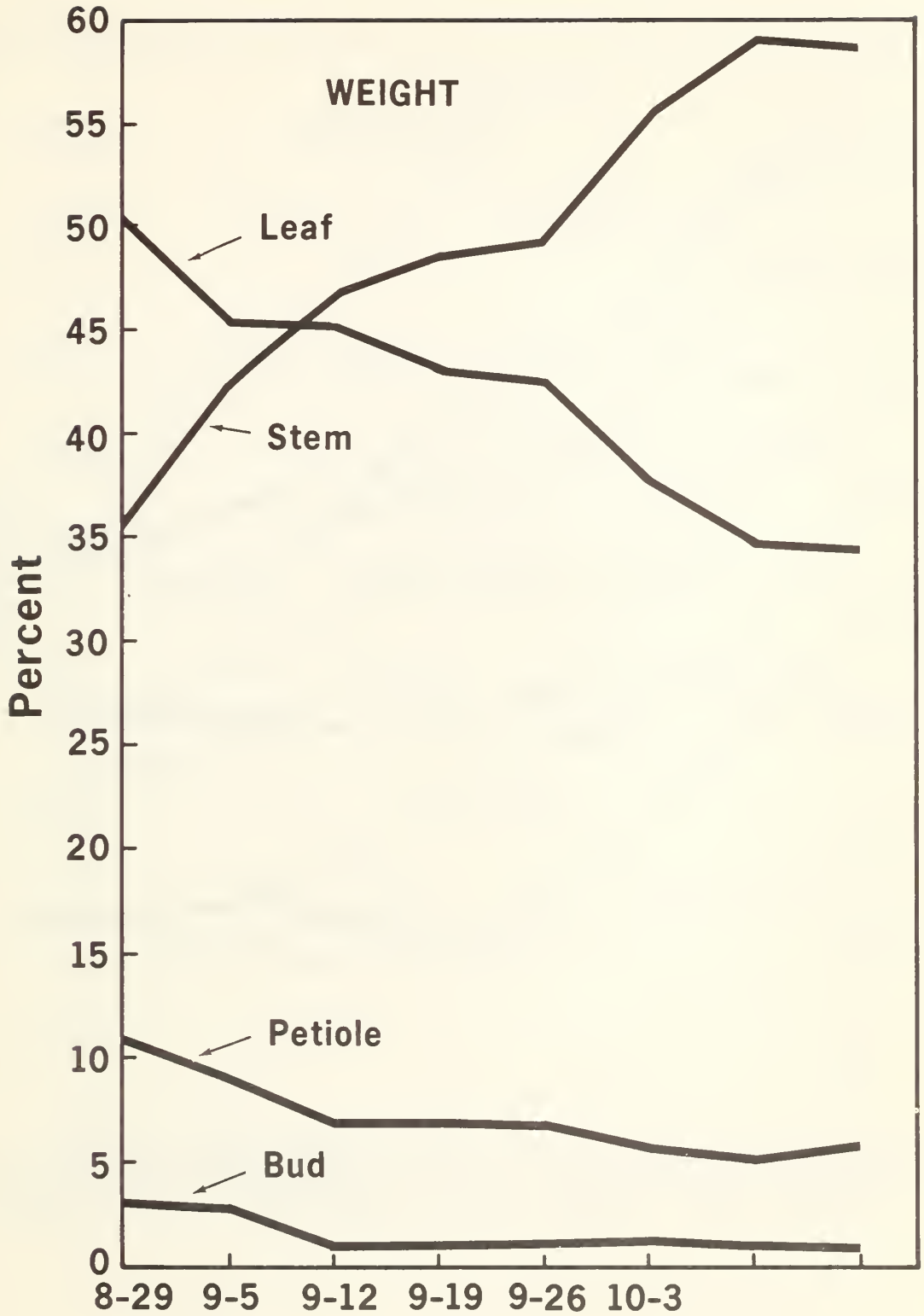

Figure 1. Weight

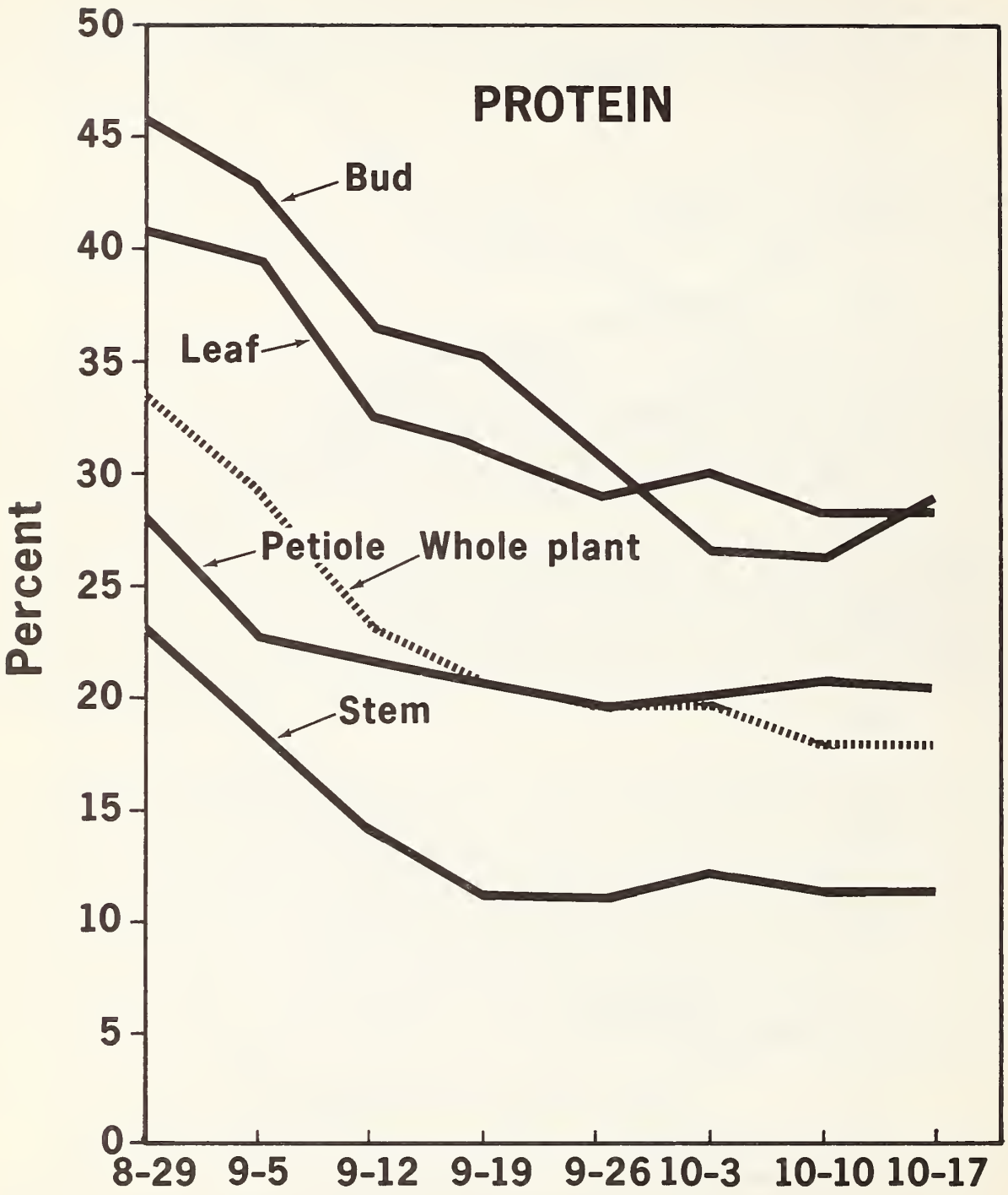

Figure 2. Protein

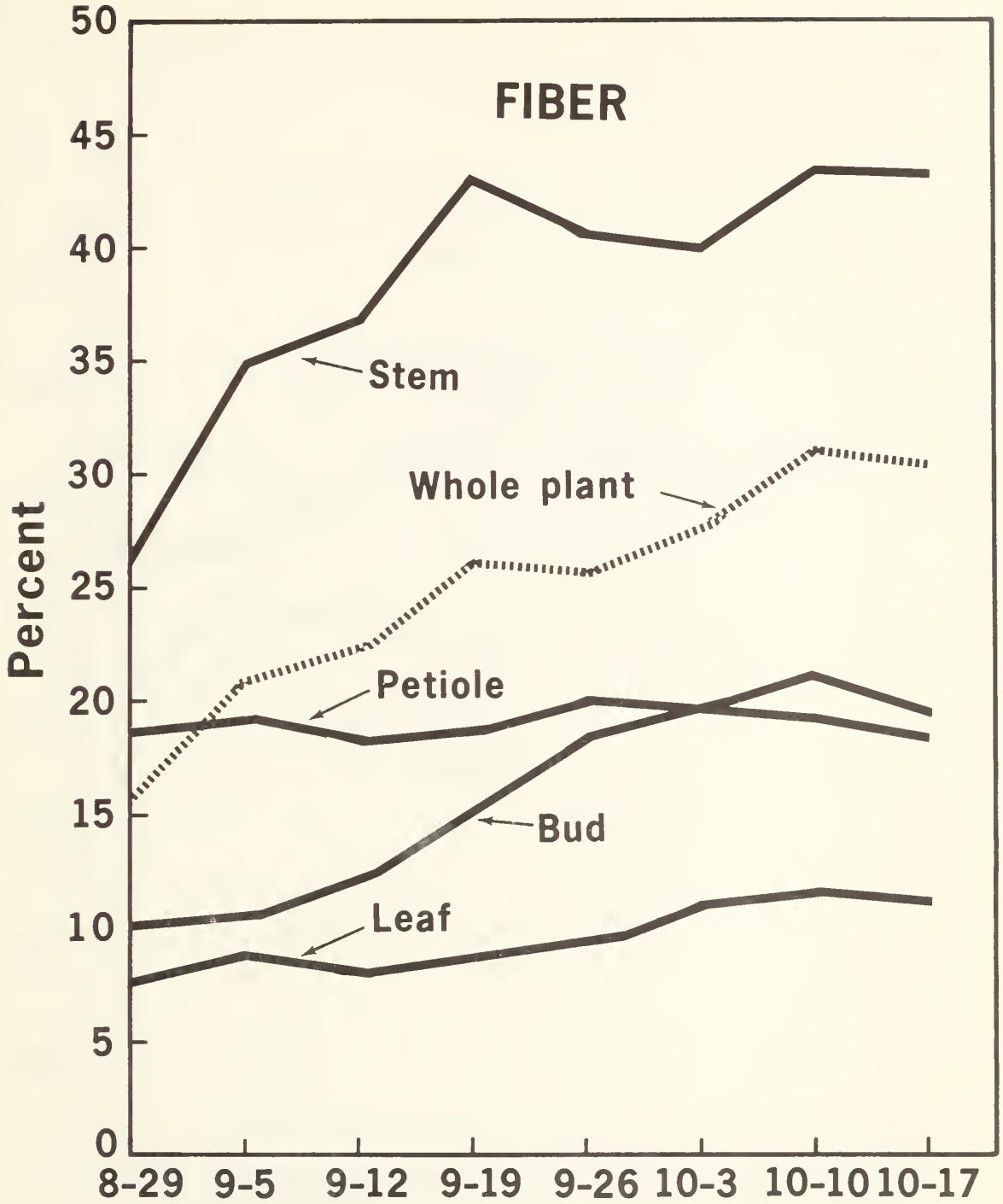

Figure 3. Fiber

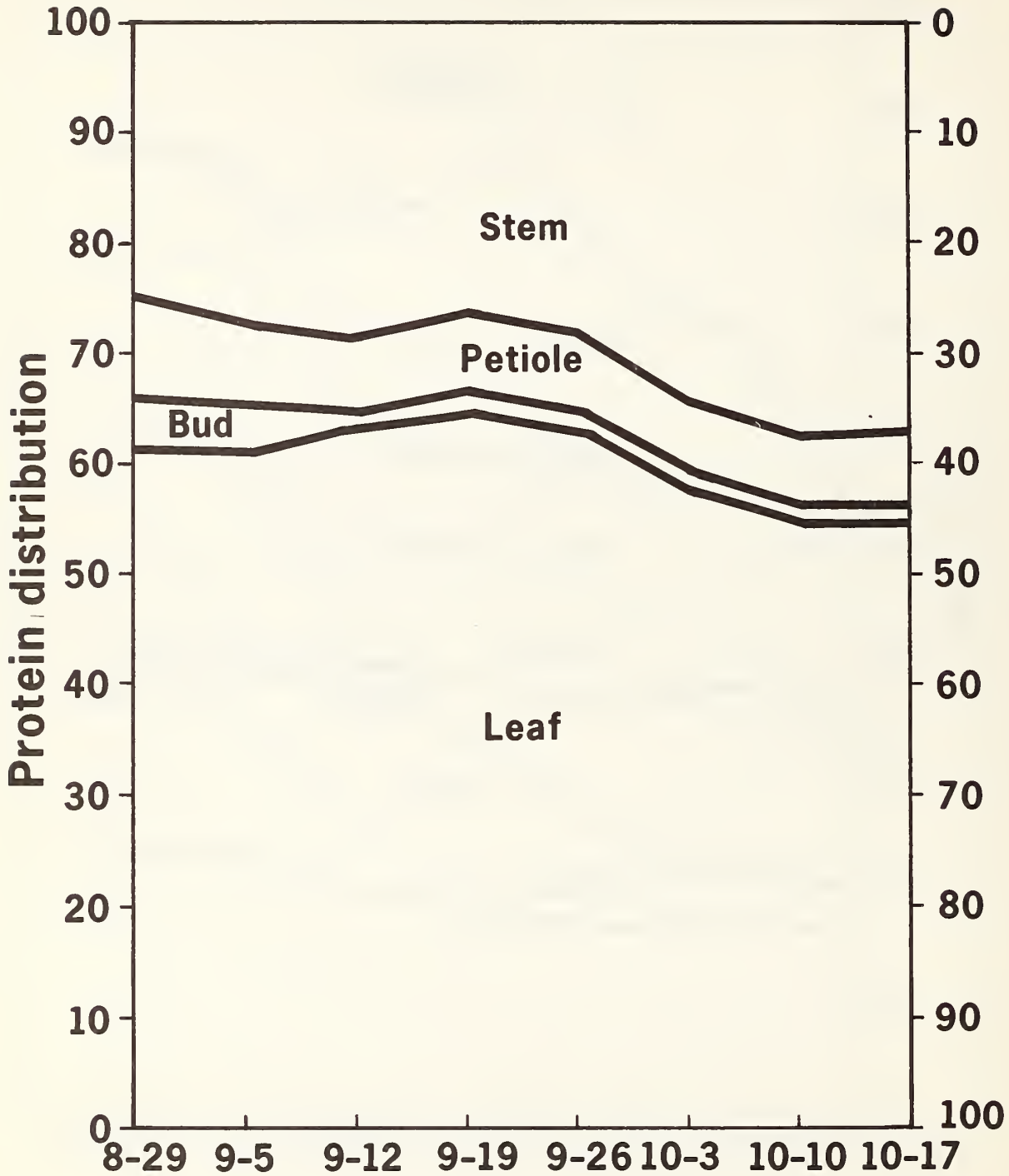

Figure 4. Protein distribution.

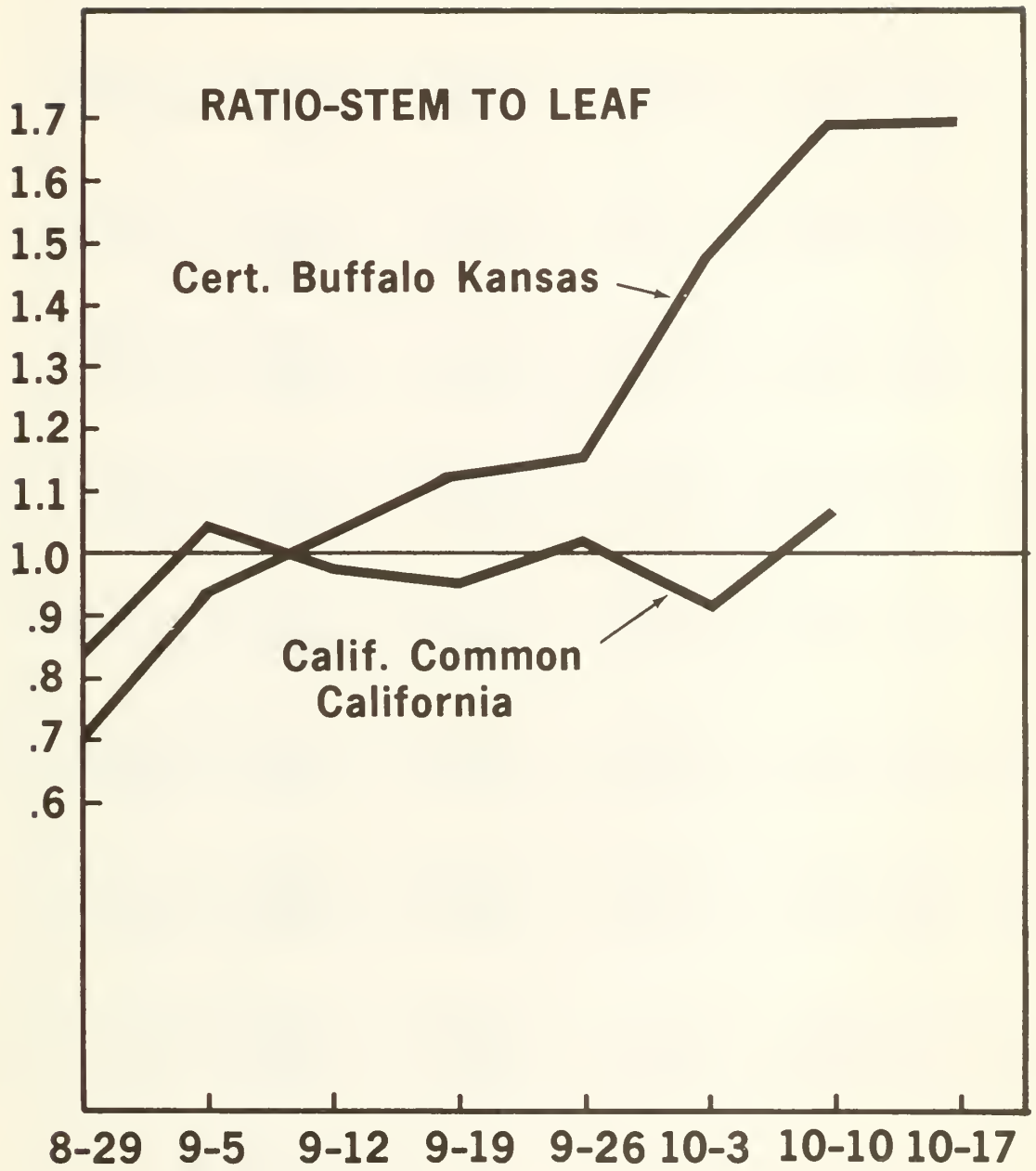

Figure 5. Ratio-stem to leaf.

Table 2. Lawrence, Kansas - Buffalo Alfalfa

	1st week 8/29/62	2nd week 9/5	3rd week 9/12	4th week 9/19	5th week 9/26	6th week 10/3	7th week 10/10	8th week 10/17	Average 8 weeks
Leaf									
Percent weight	50.25	45.36	45.17	43.24	42.63	37.65	34.83	34.62	41.72
" protein	40.93	39.63	32.56	31.06	29.13	30.25	28.25	28.31	32.52
" fiber	8.28	8.77	8.15	8.71	9.45	11.0	11.3	11.0	9.58
" ash	10.9	11.4	10.5	9.42	8.78	10.2	12.0	10.9	10.51
Buds									
Percent weight	3.13	2.86	1.00	1.07	1.16	1.28	1.16	1.00	1.58
" protein	45.81	42.69	36.63	35.25	30.94	26.69	26.25	29.00	34.16
" fiber	10.2	10.4	12.10	15.10	18.3	19.5	19.0 *	18.3	15.36
" ash	9.73	8.78	8.1	7.87	7.32	8.49	9.97	8.34	8.58
Petioles									
Percent weight	10.84	9.21	7.12	7.18	7.05	5.83	5.29	5.99	7.31
" protein	27.88	22.69	21.56	20.50	19.63	20.25	20.88	20.38	21.72
" fiber	18.62	19.2	18.30	18.7	19.9	19.5	21.0	19.4	19.33
" ash	14.57	14.08	11.4	13.9	11.2	11.4	12.5	11.4	12.56
Stem									
Percent weight	35.78	42.57	46.71	48.51	49.16	55.24	58.72	58.39	49.39
" protein	23.22	18.63	14.13	11.25	11.13	12.25	11.44	11.31	14.17
" fiber	26.26	34.9	36.70	42.9	40.7	39.9	43.7	43.2	38.53
" ash	14.19	12.3	9.1	7.58	7.64	7.41	8.01	7.73	9.25
Whole plant (computed)									
Percent protein	33.33	29.22	23.21	20.74	19.63	19.68	17.97	17.92	22.71
" fiber	15.89	20.9	22.25	26.08	25.65	27.57	31.07	30.38	24.97
" ash	12.44	11.96	9.89	8.83	8.37	8.71	9.66	9.05	9.86

* Estimated.

Table 3. Lawrence, Kansas - Buffalo Alfalfa

	1st week 8/29/62	2nd week 9/5	3rd week 9/12	4th week 9/19	5th week 9/26	6th week 10/3	7th week 10/10	8th week 10/17	Average 8 weeks
Protein									
% of total in leaf	61.71	61.53	63.37	64.76	63.25	57.88	54.77	54.70	60.25
" " " " bud	4.30	4.17	1.58	1.83	1.83	1.74	1.69	1.63	2.35
" " " " petiole	9.07	7.16	6.61	7.09	7.05	6.00	6.15	6.81	6.99
" " " " stem	24.92	27.14	28.44	26.32	27.87	34.38	37.39	36.86	30.41
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Fiber									
% of total in leaf	26.20	19.03	16.55	14.44	15.70	15.02	12.73	12.54	16.52
" " " " bud	2.00	1.42	0.54	0.62	0.83	0.91	0.71*	0.61	0.96
" " " " petiole	12.70	8.47	5.86	5.14	5.47	4.13	3.59	3.82	6.15
" " " " stem	59.10	71.08	77.05	79.80	78.00	79.94	82.97	83.03	76.37
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Ash									
% of total in leaf	44.03	43.25	47.97	46.12	44.69	44.10	43.27	41.68	44.39
" " " " bud	2.45	2.10	0.82	0.96	1.01	1.25	1.20	0.93	1.34
" " " " petiole	12.70	10.85	8.21	11.29	9.44	7.64	6.84	7.54	9.31
" " " " stem	40.82	43.80	43.00	41.63	44.86	47.01	48.69	49.85	44.96
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Weight									
% of total in leaf	50.25	45.36	45.17	43.24	42.63	37.65	34.83	34.62	41.72
" " " " bud	3.13	2.86	1.00	1.07	1.16	1.28	1.16	1.00	1.58
" " " " petiole	10.84	9.21	7.12	7.18	7.05	5.83	5.29	5.99	7.31
" " " " stem	35.78	42.57	46.71	48.51	49.16	55.24	58.72	58.39	49.39
	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

* Based on estimate.

alfalfa, unground and unclassified, were sent to one commercial firm specializing in classification by a combination of gravity and air. These people returned 13 fractions obtained at various points in their procedure. These were analyzed for protein, fiber, and ash but no fraction showed any significant improvement through increased concentration of protein, nor did any fraction show significant concentration of fiber. Other samples are currently being examined by a company engaged in electrostatic methods of separation.

So far we have been dealing here with freshly cut material from the field, with dehydrated alfalfa both ground and unground, with alfalfa hay, and with various hand and mechanical separations. Thinking that perhaps there is a possibility of making improvements at a later stage in the preparation of alfalfa for the market place, we obtained a number of samples of the commercial product sold in greatest volume in the dehydrating industry--meal made by regrinding pellets. Our interest also lay in observing how much variation occurred in this particular product as manufactured by various producers.

Eight samples were received from California, Kansas, Nebraska, Ohio, South Dakota and Washington. The Washington sample was a mixture of grass and Ladino clover. Each of these five-pound samples was accompanied by information on methods of manufacture and additives, if any. Each was carefully cut down on a riffle to obtain representative samples for proximate feed analysis and for particle size distribution analysis.

Wide variations appear in the particle size distribution as shown by the charts and tables included in this report. We will not attempt to show you all of these figures but have chosen a few to indicate what was found. Not only were sieve separations made but also sedimentation tests upon the material which passed through the 200-mesh sieve (74 microns). Three illustrations appear on logarithmatic probability paper; the coarsest, finest and an intermediate (Figure 6). You would expect the appearance of such a plotting to be a straight line if only one grinding were involved.

All screen fractions were analyzed for total solids, protein, fiber, fat and ash. Nitrogen-free extract is also tabulated. Grit was also determined on all original samples and on three of the fractions; the plus 20 mesh and the two finest fractions. Grit varied widely among the eight samples. Tables 4 to 12 are included to show the screen fractions of the several samples and proximate analyses.

Sample No. 53, (Table 9) contained 7.38 percent grit in the minus-200 mesh fraction. It might be pointed out that in every ton of this alfalfa 33 pounds of abrasive were being ground. This was enough to cause serious wear on milling machinery, piping and cyclone collectors.

Figure 7 depicts the very coarse and the two finest fractions as percentages of the whole. No. 57 (Figure 7) shows practically no material not passing 20 mesh but well over half in the very fine fractions. Conversely, No. 47 shows a little over 40 percent to be coarser than 20 mesh and less than 10 percent in the fine fractions.

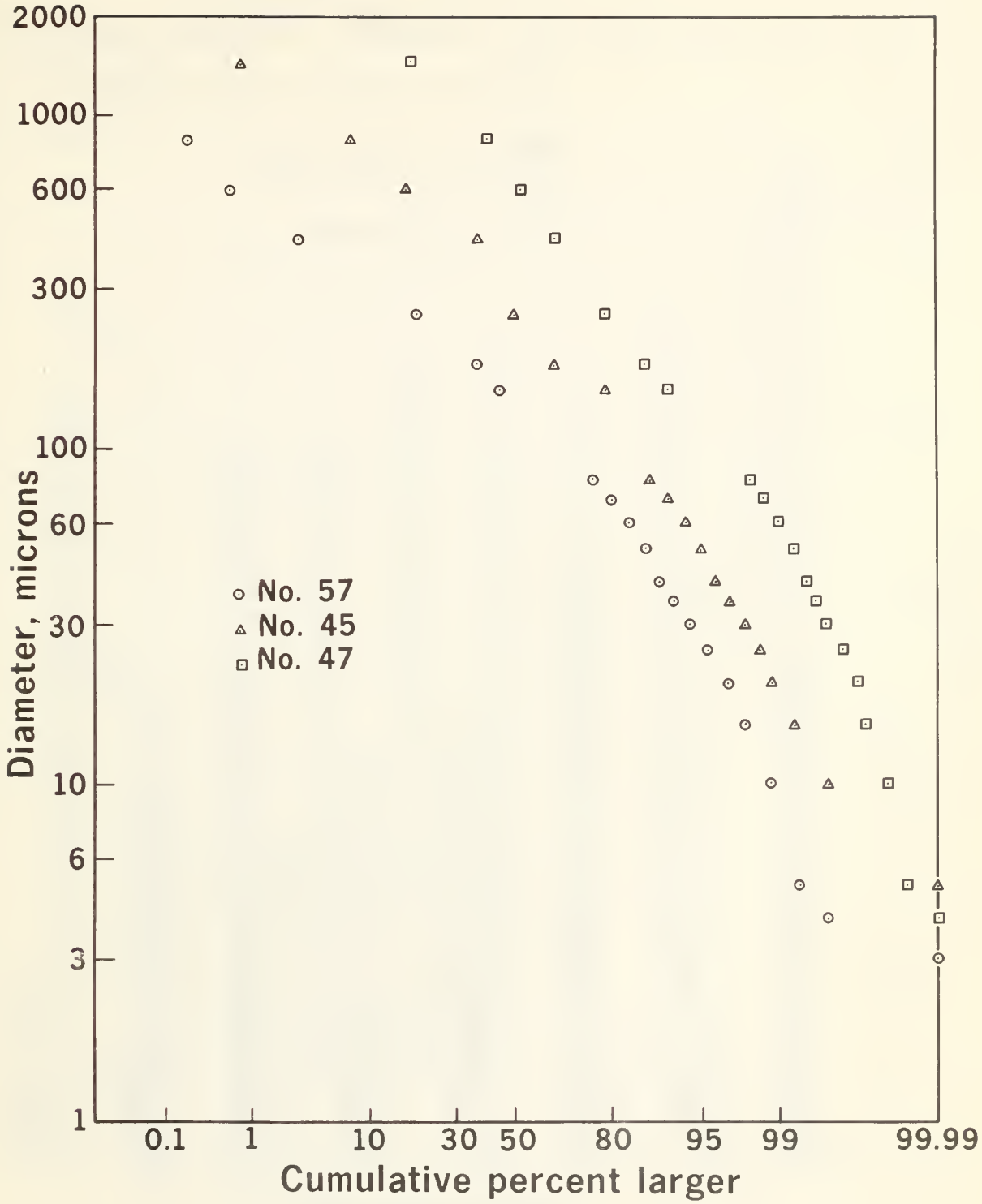

Figure 6. Cumulative percent larger.

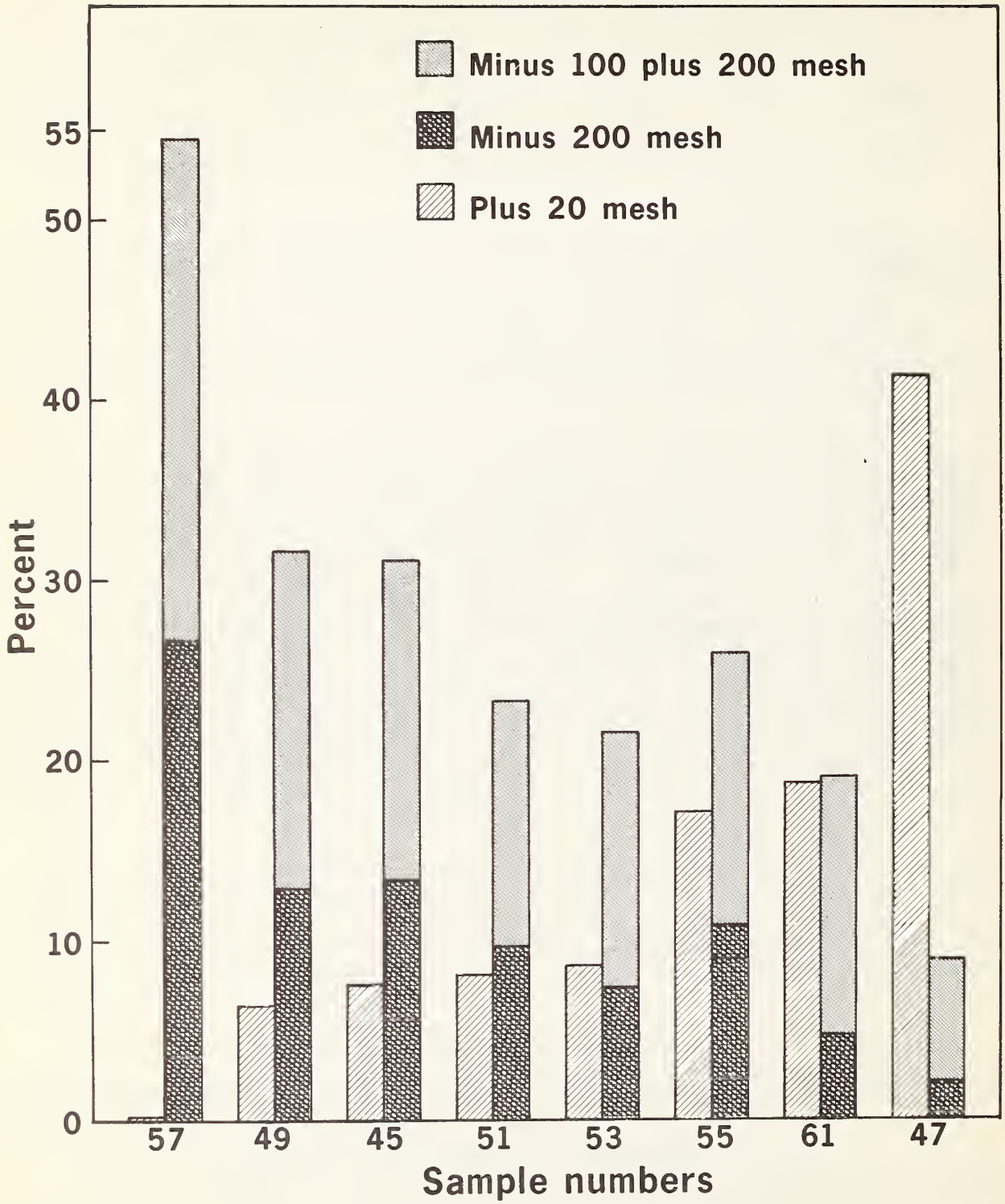

Figure 7. Sample numbers.

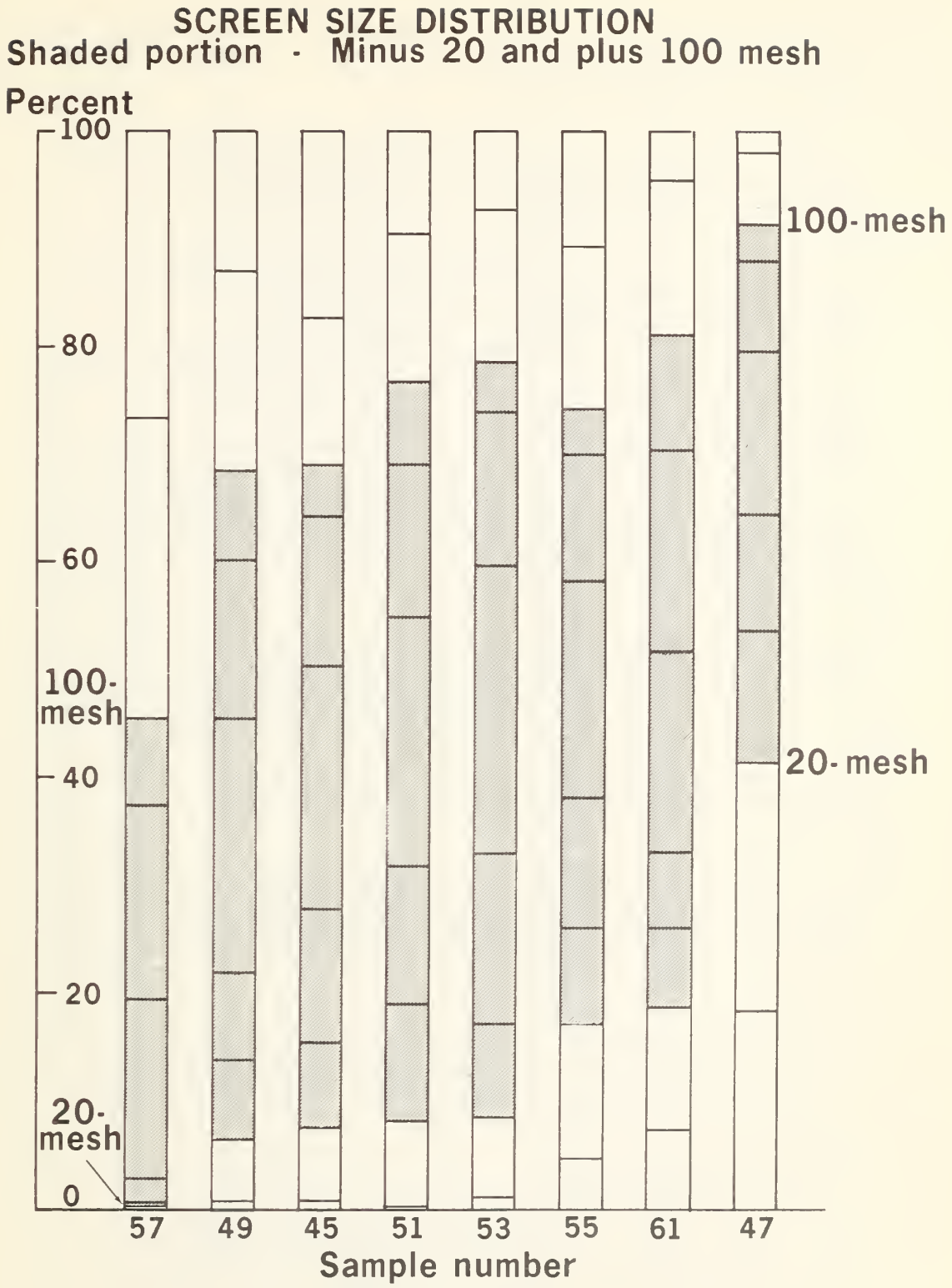

Figure 8. Screen size distribution.

Table 4. Screen analysis

Screen sizes	Sample 45	Sample 47	Sample 49	Sample 51	Sample 53	Sample 55	Sample 57	Sample 61
Percent Plus 14 mesh	0.8	18.4	0.7	0.2	1.1	4.7	0.0	7.3
" Minus 14 " 20 "	6.7	22.9	5.7	7.9	7.4	12.4	0.2	11.4
" " 20 " 30 "	7.9	12.2	7.4	10.9	8.7	8.9	0.4	7.3
" " 30 " 40 "	12.4	10.8	10.1	12.8	15.8	12.1	2.2	7.0
" " 40 " 60 "	22.5	15.2	21.6	23.0	26.6	20.0	16.6	18.5
" " 60 " 80 "	13.8	8.4	14.7	14.3	14.3	11.8	18.1	18.7
" " 80 " 100 "	4.8	3.3	8.2	7.6	4.6	4.2	8.0	10.8
" " 100 " 200 "	17.8	6.8	18.7	13.7	14.2	15.1	27.8	14.3
" " 200 "	13.3	2.0	12.9	9.6	7.3	10.8	26.7	4.7
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Feed analysis								
Total solids	93.87	94.42	92.94	93.32	92.97	91.52	93.39	
Crude protein	19.13	25.81	19.63	17.61	16.54	19.94	19.07	
Crude fiber	29.0	20.7	25.8	26.7	32.6	26.4	28.7	
Fat	3.67	6.71	5.0	4.14	2.74	4.36	3.83	
Ash	11.3	9.47	10.67	11.1	11.1	12.3	10.26	
NFE	36.90	37.31	38.90	40.45	37.02	37.00	38.14	

Table 5. Feed analysis of screen fractions (No. 388-45)

Screen sizes	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
+ 14 mesh	0.8	93.78	19.19	26.55	2.66	10.27	41.33	
- 14 + 20 "	6.7	93.72	18.94	27.53	3.17	9.81	40.55	0.63
- 20 + 30 "	7.9	93.79	17.66	29.85	2.78	9.54	40.17	
- 30 + 40 "	12.4	93.83	16.52	32.29	2.76	8.95	39.48	
- 40 + 60 "	22.5	93.89	16.58	31.31	2.76	8.83	40.52	
- 60 + 80 "	13.8	93.86	18.78	27.17	3.12	9.56	41.37	
- 80 + 100 "	4.8	93.77	20.40	25.81	3.42	9.83	40.54	
-100 + 200 "	17.8	94.50	20.17	22.65	4.05	10.11	43.02	0.88
-200 "	13.3	93.96	23.08	20.22	4.91	11.49	40.30	1.63
Weighted average	100.0	93.97	18.83	27.17	3.39	9.71	40.91	
Original sample		93.87	19.13	29.0	3.67	11.3	36.90	0.75

Original grind - 3600 rpm hammer mill 24" Dia. x 24" Length 5/64" screen opening.

Regrind of 1/4" pellets - 3600 rpm hammer mill 24" Dia. x 24" Length 8/64" screen opening.

No fat added.

Ethoxyquin added at regrinding mill.

Table 6. Feed analysis of screen fractions (No. 388-47)

	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
Screen sizes								
+ 14 mesh	18.4	94.15	26.49	19.97	5.61	9.09	38.84	
- 14 + 20 "	22.9	94.17	25.82	20.60	5.76	9.05	38.77	0.88
- 20 + 30 "	12.2	94.21	24.75	21.76	5.50	8.85	39.14	
- 30 + 40 "	10.8	94.21	24.21	22.72	5.57	8.76	38.74	
- 40 + 60 "	15.2	94.25	24.20	21.75	6.30	8.91	38.84	
- 60 + 80 "	8.4	94.25	26.33	19.20	7.13	9.04	38.30	
- 80 + 100 "	3.3	94.36	28.61	16.74	7.53	9.15	37.97	
-100 + 200 "	6.8	94.41	29.33	15.15	8.70	9.53	37.29	.75
-200 "	2.0	94.45	28.92	13.98	9.56	10.80	36.74	1.88
Weighted average	100.0	94.22	25.82	20.28	6.21	9.05	38.63	
Original sample		94.42	25.81	20.7	6.71	9.47	37.31	0.50

Original grind - 1800 rpm hammer mill 40" Dia. x 24" Length 10/64" screen opening.

Regrind of 1/4" pellets - 1200 rpm hammer mill 28" Dia. x 14" Length 10/64" screen opening.

1% fat added to pellets just ahead of regrind mill.

Ethoxyquin added in mixing chamber of pellet mill.

Table 7. Feed analysis of screen fractions (No. 388-49)

	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
Screen sizes								
+ 14 mesh	0.7	93.38	19.88	25.06	3.83	10.22	41.01	
- 14 + 20 "	5.7	93.00	19.76	25.81	3.70	10.14	40.59	0.75
- 20 + 30 "	7.4	93.09	18.33	27.39	3.94	10.22	40.12	
- 30 + 40 "	10.1	93.07	16.72	30.30	3.49	9.49	40.00	
- 40 + 60 "	21.6	93.04	16.59	29.66	3.68	9.53	40.54	
- 60 + 80 "	14.7	93.04	17.00	25.58	4.26	10.12	43.04	
- 80 + 100 "	8.2	93.18	21.20	21.25	4.82	9.83	42.90	
-100 + 200 "	18.7	93.14	22.82	18.57	5.68	10.37	42.56	1.13
-200	12.9	93.52	23.46	14.97	7.00	12.40	42.17	2.63
Weighted average	100.0	93.14	19.43	24.05	4.66	10.26	41.61	
Original sample		92.94	19.63	25.8	5.00	10.67	38.90	1.00

Original grind - 3600 rpm hammer mill 22 3/8" Dia. x 26" Length 4/64" screen opening.

Regrind of 1/4" pellets - 3600 rpm hammer mill 22 1/2" Dia. x 26" Length 8/64" screen opening.

Fat added ahead of regrind mill.

Ethoxyquin added in mixing chamber of pellet mill.

Table 8. Feed analysis of screen fractions (No. 388-51)

Screen sizes	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
+ 14 mesh	0.2)							
- 14 + 20 "	7.9)	93.69	18.28	27.22	3.38	10.67	40.45	1.00
- 20 + 30 "	10.9	93.65	17.35	29.47	3.09	9.96	40.13	
- 30 + 40 "	12.8	93.66	15.88	31.82	2.97	9.91	39.42	
- 40 + 60 "	23.0	93.61	16.09	30.45	2.98	9.82	40.66	
- 60 + 80 "	14.3	93.49	18.72	26.63	3.41	11.02	40.22	
- 80 + 100 "	7.6	93.68	20.68	21.99	4.46	10.67	42.20	
-100 + 200 "	13.7	93.64	22.63	19.12	5.06	11.21	41.98	.88
-200 "	9.6	93.84	22.98	16.30	5.84	13.43	41.45	4.75
Weighted average	100.00	93.64	18.66	26.16	3.76	10.69	40.74	
Original sample		93.32	17.61	26.7	4.14	11.1	40.45	0.75

Original grind - 3600 rpm hammer mill 27" Dia. 4/64" screen opening.

Regrind of 1/4" pellets - 1750 rpm hammer mill 27" Dia. 4/64" screen opening.

1/2% Fat added to pellets just prior to regrinding.

Ethoxyquin added in mixing chamber of pellet mill.

Table 9. Feed analysis of screen fractions (No. 388-53)

	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
Screen sizes								
+ 14 mesh	1.1	93.49	16.51	30.91	3.05	10.51	39.02	
- 14 + 20 "	7.4	93.18	16.16	32.52	2.50	10.55	38.27	2.00
- 20 + 30 "	8.7	93.33	15.27	34.72	2.45	9.75	37.81	
- 30 + 40 "	15.8	93.27	14.54	38.38	2.34	9.18	35.56	
- 40 + 60 "	26.6	93.26	15.41	35.81	2.26	9.40	37.12	
- 60 + 80 "	14.3	93.20	17.30	31.44	2.53	11.16	37.57	
- 80 + 100 "	4.6	93.49	18.58	28.24	2.71	12.51	37.96	
-100 + 200 "	14.2	93.40	19.94	25.05	3.16	13.81	38.04	4.50
-200 "	7.3	93.58	19.97	23.94	3.00	15.71	37.38	7.38
Weighted average	100.0	93.31	16.72	32.46	2.56	10.97	37.29	
Original sample		92.97	16.54	32.6	2.74	11.1	37.02	1.63

Original grind - 1750 rpm hammer mill 49" Dia. x 24" Length 8/64" screen opening.

Regrind of 1/4" pellets - 3600 rpm hammer mill 30" Dia. x 22" Length 8/64" screen opening.

Table 10. Feed analysis of screen fractions (No. 388-55)

	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
Screen sizes								
+ 14 mesh	4.7	92.03	20.24	25.54	3.60	13.69	36.93	
- 14 + 20 "	12.4	92.09	20.16	26.60	3.45	12.27	37.52	3.00
- 20 + 30 "	8.9	92.22	19.18	29.28	3.55	11.60	36.39	
- 30 + 40 "	12.1	91.93	17.68	31.65	3.10	10.01	37.56	
- 40 + 60 "	20.0	91.81	17.77	31.70	3.63	10.08	36.82	
- 60 + 80 "	11.8	92.02	19.63	27.17	4.12	11.19	37.89	
- 80 + 100 "	4.2	92.29	21.81	23.73	4.49	12.35	37.62	
-100 + 200 "	15.1	92.35	23.55	20.79	5.09	12.89	37.68	3.00
-200 "	10.8	92.60	23.15	19.87	5.72	13.71	37.55	4.00
Weighted average	100.0	92.12	20.14	26.76	4.08	11.69	37.33	
Original sample		91.52	19.94	26.4	4.36	12.3	37.00	2.94

Original grind - 3600 rpm hammer mill 24" Dia. (Alternate 1750 rpm 36" Dia.) 3/64" screen opening.

Regrind of 1/4" pellets - 1750 rpm hammer mill 24" Dia. 8/64" screen opening.

1/2% fat added while regrinding.

Ethoxyquin added in pellet mill mixing chamber.

Table 11. Feed analysis of screen fractions (No. 388-57)

Screen sizes	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
+ 14 mesh	---)							
- 14 + 20 "	0.2)							
- 20 + 30 "	0.4)	93.58	14.16	40.61	2.43	7.57	35.23	
- 30 + 40 "	2.2	93.55	11.36	44.36	2.16	6.52	35.60	0.25
- 40 + 60 "	16.6	93.46	11.64	41.94	2.13	7.12	37.17	
- 60 + 80 "	18.1	93.47	15.31	34.66	2.47	8.56	39.00	
- 80 + 100 "	8.0	93.31	19.49	27.11	3.29	9.26	40.85	
-100 + 200 "	27.8	93.37	22.76	22.38	3.99	9.67	41.20	.38
-200 "	26.7	93.46	23.07	20.33	4.07	11.56	40.97	1.25
Weighted average	100.0	93.43	19.08	28.27	3.32	9.44	39.88	
Original sample		93.39	19.07	28.7	3.83	10.26	38.14	0.50

Original grind - 1700 rpm hammer mill 74 3/4" Dia. x 28 1/2" Length screen opening 8/64" (sifted through 24 mesh).

Regrind of 1/4" pellets - 3600 rpm hammer mill 15 1/2" Dia. x 28 1/2" Length screen opening 8/64"

1% fat added.

Ethoxyquin added.

Table 12. Feed analysis of screen fractions (No. 388-61)

	% Weight	% Total solids	% Crude protein	% Crude fiber	% Fat	% Ash	% NFE	% Grit
Screen sizes								
+ 14 mesh	7.34	93.3	17.63	22.3	5.26	10.93	43.88	
- 14 + 20 "	11.36	93.4	18.00	22.6	5.29	10.92	43.19	0.38
- 20 + 30 "	7.34	93.6	17.81	22.4	4.97	10.58	44.24	
- 30 + 40 "	7.04	93.1	17.31	22.6	4.84	10.40	44.85	
- 40 + 60 "	18.49	93.4	16.75	23.3	4.37	10.30	45.28	
- 60 + 80 "	18.69	93.5	16.63	24.9	4.51	10.63	43.33	
- 80 + 100 "	10.75	93.6	18.31	23.7	5.02	10.79	42.18	
-100 + 200 "	14.27	93.4	20.06	21.1	5.77	10.92	42.15	0.38
-200 "	4.72	93.6	21.19	18.2	6.82	11.22	42.57	1.38
Weighted average	100.0	93.4	17.90	22.8	5.03	10.69	43.58	
Original sample		93.1	17.73	23.1	5.05	10.96	43.16	

Original grind - 3600 rpm hammer mill 24" Dia. x 24" Length screen opening either 6/64" or 7/64".

Regrind of 1/4" pellets - 3600 rpm hammer mill 24" dia. x 20" Length 12/64" screen opening

No fat or antioxidant added.

We have attempted in Figure 8 to show graphically where the best fractions lie in each sample. Perhaps both 57 and 47 are serving a particular market and, hence, need the very fine and the very coarse, respectively. Numbers 51 and 53 have the greatest amounts passing through 20 mesh and yet not so fine as to pass through 100 mesh.

In conclusion, the turbomill has been repaired and returned and we anticipate further fine milling, air classification and all attendant testing. When fresh alfalfa is again available, we want to continue our work of fractionation on the freshly dehydrated material. We are expecting delivery near the beginning of a new crop of a small dehydrator for experimental uses and this will afford the opportunity to put some of our experimental work on a commercial or semi-commercial scale.

RECENT AGRONOMIC ADVANCES IN ALFALFA PRODUCTION

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I am pleased to summarize recent advances in alfalfa production and have interpreted your interests to be centered largely around new varieties and cultural practices. My report deals largely with those topics, but I will allude to more basic kinds of research which are the foundation for continued progress in applied research. Alfalfa is grown on about 30 million acres throughout most of the United States. Consequently, the crop is subjected to a wide range of climatic, disease, and insect conditions. Choosing an adapted variety and planting good seed are prerequisites to profitable production.

New Varieties

The varieties described herein were released during the last 2 years. It is anticipated that some certified seed of each, except Travois, will be marketed in 1963. All except Travois are good varieties in their respective areas of adaptation for producing dehydrated alfalfa products. Travois is best adapted for grazing.

Cayuga. Cayuga is a bacterial wilt-resistant variety which does well under intensive management on good alfalfa soils in the Northeastern States. It is intermediate in recovery growth rate between Du Puits and Vernal. Seedling vigor of Cayuga was less and first-year hay yields averaged one-half

ton lower than Du Puits; second-year production was equal. Third- and fourth-year yields, however, were greatly in favor of Cayuga, because stands of Du Puits declined.

Cherokee. This variety has moderate winter hardiness and is adapted to North Carolina and probably to neighboring States. It yields well and has more resistance to leafhopper yellowing and rusts than other varieties. It is also slightly more persistent than other varieties. Early spring growth behavior and rapidity of recovery after cutting are intermediate between those of Atlantic and Du Puits. Cherokee is moderately susceptible to bacterial wilt.

Uinta. In Utah tests, Uinta was more resistant to yellow leaf blotch and downy mildew than Ranger and equal in resistance to bacterial wilt. During a 4-year period, Uinta produced 67 percent more seed than Ranger alfalfa. Tests in Utah and in several Western and Central States indicated that Uinta alfalfa was equal to or slightly better than Ranger in hay yield. In winter hardiness, the new variety is intermediate between Ranger and Buffalo. It is adapted to parts of the intermountain region of western United States with climatic conditions similar to those of Logan, Utah.

Travois. Perhaps the newest alfalfa is Travois, a product of many years of selection from several hardy, root-proliferating alfalfa strains at South Dakota State College, Brookings. According to M. D. Rumbaugh, Travois is well adapted to the severe climate of the Northern Great Plains, where cold winters with inadequate snow cover are common and annual precipitation is less than 25 inches. Because of its ability to produce stem buds on laterally spreading roots and its lower forage yields, Travois should be used only in permanent or semi-permanent pastures or rangeland grazing areas. Travois is equal or superior to Vernal alfalfa in winter hardiness and resistant to bacterial wilt and certain foliar diseases.

Sonora. Sonora is a new winter-tender spotted alfalfa aphid-resistant alfalfa variety developed by alfalfa workers in the Southwest. The research group is composed of scientists from the Agricultural Experiment Stations of Arizona, California, and Nevada, and the Crops and Entomology Research Divisions of the Agricultural Research Service, U. S. Department of Agriculture. Resources were pooled in 1957 to breed an alfalfa variety resistant to the spotted alfalfa aphid. Moapa alfalfa, which virtually saved the alfalfa industry in Arizona, California, and southern Nevada from complete destruction by the spotted aphid, was the first product of this group.

Sonora is similar to Moapa in resistance to the spotted alfalfa aphid and to the two species of southern root-knot nematodes. It has consistently yielded more than Moapa during winter and early spring in Arizona and in the Imperial Valley of California. Sonora and Moapa did not differ significantly in season total hay at Logandale, Nevada, but the seasonal distribution of yield was slightly different. Sonora yielded slightly more in the final harvest of the season, which represented growth between mid-October and the first week in December. The importance to the grower of superior seedling growth and winter growth under short days will probably determine whether he should choose Sonora or Moapa in the deep Southwest. Moapa may be slightly more persistent.

Varieties developed by industry. Several varieties have been released by industrial firms that have initiated research on evaluation and breeding of alfalfa. I am less familiar with performance characteristics of varieties from industry and will identify them only as to name and probable area of adaptation. Three of the new varieties are given below; each was reviewed by the National Certified Alfalfa Variety Review Board, in addition to Cayuga, Uinta, Cherokee, and Sonora. The varieties from industry are as follows:

Variety name	Area of adaptation indicated by originator
525	"Similar to that of Vernal and Ranger"
Progress	"Vernal and Ranger areas"
WL 202	"Eastern Nebraska and northern Missouri north to Canadian line and east to Appalachian Mountains"

National Certified Alfalfa Variety Review Board Established

The establishment of this Review Board in 1961 was one of the recommendations of the Joint Alfalfa Work Conference. The Conference consisted of representatives from the three regional Alfalfa Improvement Conferences, the American Seed Trade Association, the State seed certifying agencies, the USDA Agricultural Research Service, and the State Agricultural Experiment Station Directors.

The functions of the new Board are to review and evaluate information in requests submitted by public or private agencies for producing certified seed of new alfalfa varieties. The Board uses criteria developed by the Joint Alfalfa Work Conference and State seed certifying agencies for judging the merits of an application and reports to the certifying agencies. Thus, the Board serves in an advisory capacity. Public and private agencies each have two voting members on the Board.

The Review Board helps to bridge a gap in a changing alfalfa situation in which private industry has joined public agencies in the development of new varieties. Buying certified seed is one of the best assurances of obtaining seed true to variety name, regardless of whether the variety was developed by a public or a private agency.

Breeding for Disease Resistance

Most of the new alfalfa varieties have more resistance to some of the foliar diseases than was available in the old line varieties. Greater disease resistance can be expected to result in improved quality.

Several new techniques for increasing the efficiency of screening for resistance to different diseases have been published and will speed up selection for resistance to the diseases involved. A considerable body of data was developed on the genetics and pathology of black stem diseases of alfalfa in the North Central States through the regional project NC-37 (13).

While considerable advance has been made in improving the quality, persistence, and yield of alfalfa by incorporating disease resistance, improved

disease resistance continues to be a very challenging area of research. Improved resistance to any one of about 15 diseases caused by bacteria, fungi, or viruses would improve quality and performance of alfalfa.

Research on Verticillium Wilt of Alfalfa

A grant to support the development of strains of alfalfa that will resist Verticillium wilt disease was made to the University of Perugia, Perugia, Italy. The grant, which is administered by the Foreign Research and Technical Programs Division of USDA's Agricultural Research Service, allows 5 years of research aimed at obtaining seed of resistant lines of alfalfa for possible use in the United States. The program is financed by foreign currencies obtained from the sale abroad of U. S. agricultural commodities under Public Law 480.

Verticillium wilt is causing severe losses in European alfalfa fields. Although the disease has not yet attacked alfalfa in this country, United States varieties tested in Europe were susceptible. Neither public nor private agencies in the United States are conducting research on the strain of this fungus that attacks alfalfa, because of the danger of accidental introduction of the disease into alfalfa-producing areas. Imported alfalfa seed from Europe is now being treated with a suitable fungicide under supervision of plant quarantine officials to prevent introduction of the disease.

Breeding for Insect Resistance

Pea aphid. Localized pea aphid infestations are common over most of the United States, and sporadic heavy infestations may destroy an entire spring growth. Since pea aphid damage to alfalfa is not always conspicuous, damage may be more extensive than generally realized. Yields of alfalfa at Hays, Kansas, were increased 151 percent by control of pea aphids with insecticides (5).

Pea aphid-resistant plants have been recovered from many varieties of alfalfa evaluated (3, 6, 9). In general, Turkistan derivatives were good sources of resistance. Several selection criteria were found to be effective in isolating pea aphid-resistant plants (10). It was possible to select and evaluate plants for resistance at all stages of growth and to demonstrate that the resistance factor was present at the early cotyledon stage. Several experimental synthetics with resistance to pea and spotted alfalfa aphids and bacterial wilt have been developed in several States and are now being evaulated for agronomic characters.

Potato leafhopper. The following experiment in Wisconsin (11) sheds light on the toll taken by the potato leafhopper, Empoasca fabae (Harris). Plots were laid out so that one set was sprayed to control insects and another was not. The varieties used were Vernal and Narragansett. The average yields over all treatments and years were 4.69 tons of hay and 0.94 ton of protein per acre for the sprayed conditions, and 4.17 tons of hay and 0.81 ton of protein per acre per year under insect infestations. In another Wisconsin experiment (12), leafhoppers reduced the quantity of protein, ash, phosphorus, and calcium--constituents important in animal nutrition--more than they reduced

hay yields. Research in progress indicates that it should be possible to breed resistant varieties which will control most damage from the leafhopper.

Alfalfa weevil. The most striking advance in alfalfa production during the past several years in eastern United States is the use of fall applications of granular heptachlor to control the alfalfa weevil. In North Carolina, for example, nearly 100 percent of the alfalfa acreage is being treated between October 15 and November 15. It is probably safe to say that this practice saved alfalfa production in North Carolina. Finding that fall applications were effective can be credited to North Carolina experiment station entomologist W. V. Campbell.

Interaction of Variety and Cutting Date
(From L. J. Elling, University of Minnesota)

Minnesota studies in 1961 and 1962 on cutting dates indicated the importance of harvesting alfalfa varieties in accordance with their respective stages of growth. Seven varieties, 2 early maturing, 3 intermediate, and 2 late, were studied. Cutting all varieties at the same time favored the early-maturing. Cutting each at its optimum time gave the later-maturing varieties an even opportunity to produce. As in previous studies of this kind, yield increased with stage of growth, but quality of hay decreased. This study also emphasized the importance of conducting variety trials in such a manner that each variety is cut "when ready" instead of all the same day, particularly when varieties differ in time of maturity.

Variation in Coumestrol Content of Alfalfa Studied

A study of the causes and nature of variation of coumestrol content of alfalfa (to be published by C. H. Hanson, G. O. Kohler, and coworkers) was undertaken soon after the Federal Food and Drug Administration limited the use of the synthetic estrogen diethylstilbestrol. For the present study, five varieties of alfalfa were harvested at the 1/10-bloom stage in each of 2 years (three cuttings per year) at University Park, Pennsylvania; Raleigh, North Carolina; Lincoln, Nebraska; Manhattan, Kansas; Ames, Iowa; Logan, Utah; and Davis, California. At Lincoln, Davis, and University Park, two varieties were also harvested at six stages of growth ranging from 10 inches high to 25 days after full bloom. Coumestrol determinations were made at the Western Regional Research Laboratory, Albany, California.

Locations, cuttings, years, and their interactions were important sources of variation affecting coumestrol content of alfalfa cut at the 1/10-bloom stage. The location effect was large; coumestrol content ranged from an average of about 12 ppm at Logan and Davis to an average of 126 ppm at Ames (dry-weight basis). Stage of growth was also important. At Lincoln, coumestrol generally increased in quantity with successive stages of growth, reaching the highest level 25 days after full bloom. Increased coumestrol, however, was offset by lower protein and higher crude fiber contents. At Davis, most samples were low. The information obtained will be useful for making more effective use of this naturally occurring estrogen.

The effects of specific environmental factors on coumestrol activity are being studied in temperature- and light-controlled growth chambers at Brookings, South Dakota.

Hybrid Alfalfa

Progress has been made in developing high-yielding two-clone combinations. In Nevada (2), the best two-clone experimental combinations yielded 18 to 24 percent more than the check varieties. Equally promising yields from two-clone combinations have been obtained in Arizona, Nebraska, California, Indiana, and other States. While significant gains in yield have been made in first-generation alfalfa hybrids, problems remain.

Time does not permit discussion of research in alfalfa genetics, the science upon which alfalfa breeding is based. Much effort is being devoted to obtaining maximum utilization of hybrid vigor (4, and others) to studying the effect of generations of seed increase on synthetic or hybrid performance (8), and to numerous other facets of heterosis.

Alfalfa presents pollination problems in seed production which tax the ingenuity of the plant breeder. Recent studies indicate that honey bees do not always provide the random pollination which has been assumed in producing seed of hybrid combinations. The bees may establish foraging patterns and work only one clone of a combination (1), resulting in self-pollination and loss in vigor (7).

We can look with pride on progress made, but we have a long way to go before fully exploiting this valuable legume. We have yet to put the first hybrid alfalfa on the market. Resistance to many diseases and insect pests is needed. Much needs to be done to improve and stabilize quality. Advances in any of these areas will increase the competitiveness of alfalfa products.

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